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Karaikudi – 630 003

DIRECTORATE OF DISTANCE EDUCATION

M.Sc., (Chemistry)

IV-SEMESTER

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Analytical Chemistry

SYLLABI-BOOK MAPPING TABLE
CURRICULUM AND INSTRUCTION

CONTENTS

BLOCK-I
ERROR ANALYSIS

Unit 1: ERRORS IN CHEMICAL ANALYSIS

Structure

1.1 INTRODUCTION

Any measurement is limited by the precision of the measuring instruments and the technique and the skill of the observer. Where a measurement consists of a single reading on a simple piece of laboratory equipment, for example a burette or a thermometer, one would expect the number of variables contributing to uncertainties in that measurement to be fewer than a measurement which is the result of a multi-step process consisting of two or more weight measurements, a titration and the use of a variety of reagents. It is important to be able to estimate the uncertainty in any measurement because not doing so leaves the investigator as ignorant as though there were no measurement at all. This unit 1 giving you the basic knowledge about error and its classifications in chemical analysis.

1.2 OBJECTIVES

After going through this unit, you will be able to

- ❖ To know the basic of error analysis
- ❖ To know the classification of errors
- ❖ To understating the concept of systematic and random errors
- ❖ To defer the absolute and relative.

1.3 ERRORS

- ➢ Measurements invariably involve errors and uncertainties.
- ➢ It is impossible to perform a chemical analysis that is totally free of errors or uncertainties.
- ➢ We can only hope to minimize errors and estimate their size with acceptable accuracy.
- ➢ Errors are caused by faulty calibrations or standardizations or by random variations and uncertainties in results.

- Frequent calibrations, standardizations, and analyses of known samples can sometimes be used to lessen all but the random errors and uncertainties.

The term error has two slightly different meanings.

1. Error refers to the difference between a measured value and the "true" or "known" value.
2. Error often denotes the estimated uncertainty in a measurement or experiment.

1.4 CLASSIFICATIONS OF ERRORS

Chemical analyses are affected by at least two types of errors such as *systematic* (or *determinate*) *error* and *Random* (or *indeterminate*) *error*.

- *Systematic* (or *determinate*) *error*, causes the mean of a data set to differ from the accepted value. Examples; glassware, instrumentation and etc.
- *Random* (or *indeterminate*) *error*, causes data to be scattered more or less symmetrically around a mean value. Examples; environment, analyst and etc.

A third type of error is *gross error*

- These differ from indeterminate and determinate errors.
- They usually occur only occasionally, are often large, and may cause a result to be either high or low.
- They are often the product of human errors.
- Gross errors lead to outliers, results that appear to differ markedly from all other data in a set of replicate measurements.

1.5 SYSTEMATIC ERROR

Systematic errors have a definite value, an assignable cause, and the same magnitude for replicate measurements made in the same way. They lead to bias in measurement results. There are three types of systematic errors:

i. Instrumental errors
ii. Method errors
iii. Personal errors

a) Instrumental errors

- ✓ Instrumental errors are caused by nonideal instrument behavior, by faulty calibrations, or by use under inappropriate conditions.
- ✓ Pipets, burets, and volumetric flasks may hold or deliver volumes slightly different from those indicated by their graduations.
- ✓ Calibration eliminates most systematic errors of this type.
- ✓ Electronic instruments can be influenced by noise, temperature, pH and are also subject to systematic errors.
- ✓ Errors of these types usually are detectable and correctable.

b) Method errors

- ✓ The nonideal chemical or physical behaviour of the reagents and reactions on which an analysis is based often introduce systematic method errors.
- ✓ Such sources of nonideality include the slowness of some reactions, the incompleteness of others, the instability of some species, the lack of specificity of most reagents, and the possible occurrence of side reactions that interfere with the measurement process.
- ✓ Errors inherent in a method are often difficult to detect and hence, these errors are usually the most difficult to identify and correct.

c) Personal errors

- ✓ Result from the carelessness, inattention, or personal limitations of the experimenter.
- ✓ Many measurements require personal judgments.
- ✓ Examples include estimating the position of a pointer between two scale divisions, the colour of a solution at the end point in a titration, or the level of a liquid with respect to a graduation in a pipet or buret.
- ✓ Judgments of this type are often subject to systematic, unidirectional errors.
- ✓ A universal source of personal error is prejudice, or bias.
- ✓ Number bias is another source of personal error that varies considerably from person to person.
- ✓ The most frequent number bias encountered in estimating the position of a needle on a scale involves a preference for the digits 0 and 5.
- ✓ Also common is a prejudice favoring small digits over large and even numbers over odd.
- ✓ Digital and computer displays on pH meters, laboratory balances, and other electronic instruments eliminate number bias because no judgment is involved in taking a reading.

1.6 RANDOM ERROR

The errors which remain even after the systematic errors have been taken care of is known as residual or random errors. *Random errors* arise due to the large number of small factors that change from one measurement to another. The changes may be sometimes such that we are not aware. These changes or disturbances are lumped together and are called random or residual.

1.7 ADDITIVE AND PROPORTIONAL ERRORS

Systematic errors may be either additive error or proportional error.

- ➢ *Additive error* does not depend on constituent present in the determination e.g. loss in weight of a crucible in which a precipitate is ignited.
- ➢ *Proportional error* depends on the amount of the constituent e.g. impurities in standard compound

Proportional errors decrease or increase in proportion to the size of the sample. A common cause of proportional errors is the presence of interfering contaminants in the sample. For example, a widely used method for the determination of copper is based on the reaction of copper(II) ion with potassium iodide to give iodine. The quantity of iodine is then measured and is proportional to the amount of copper. Iron(III), if present, also liberates iodine from potassium iodide. Unless steps are taken to prevent this interference, high results are observed for the percentage of copper because the iodine produced will be a measure of the copper(II) and iron(III) in the sample.

The size of this error is fixed by the fraction of iron contamination, which is independent of the size of sample taken. If the sample size is doubled, for example, the amount of iodine liberated by both the copper and the iron contaminant is also doubled. Thus, the magnitude of the reported percentage of copper is independent of sample size.

1.8 ABSOLUTE AND RELATIVE ERRORS

Absolute error

The absolute error of a measurement is the difference between the measured value (x_i) and the true value (x_t). If the measurement result is low, the sign is negative; if the measurement result is high, the sign is positive. Example: Absolute error in the micro-Kjeldahl determination of nitrogen (Figure 1.8.1).

$$E_a = x_i - x_t$$

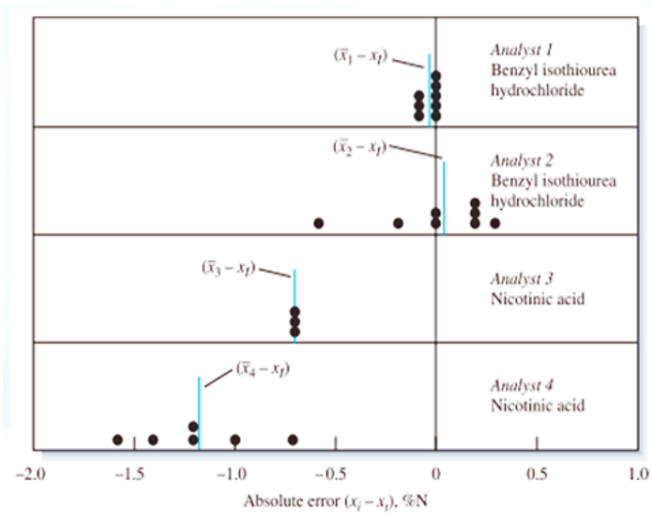


Figure 1.8.1: Absolute error in the micro-Kjeldahl determination of nitrogen

Relative error

The relative error of a measurement is the absolute error divided by the true value (x_t). Relative error may be expressed in percent, parts per thousand, or parts per million (ppm), depending on the magnitude of the result.

$$E_r = \frac{E_a}{x_t} \times 100\%$$

Check Your Progress – 1

1. What are the term in error?
2. Define systematic error?
3. What is random error?
4. Write the type of systematic error?
5. What is additive error?

1.9 ANSWER TO CHECK YOUR PROGRESS QUESTION

1. The term error has two slightly different meanings.
   - ✓ Error refers to the difference between a measured value and the “true” or “known” value.
   - ✓ Error often denotes the estimated uncertainty in a measurement or experiment**.**
2. Systematic (or determinate) error, causes the mean of a data set to differ from the accepted value.
3. Random (or indeterminate) error, causes data to be scattered more or less symmetrically around a mean value.
4. There are three types of systematic errors:
   - ✓ Instrumental errors
   - ✓ Method errors
   - ✓ Personal errors
5. Additive error does not depend on constituent present in the determination e.g. loss in weight of a crucible in which a precipitate is ignited

1.10 SUMMARY

In summary, unit 1 briefly discussed about the errors in chemical analysis. More briefly it deals about types of errors such as systematic error, random error, additive error, and proportional error, absolute and relative errors.

1.11 KEY WORDS

- ❖ Error
- ❖ Systematic error
- ❖ Random error
- ❖ Additive and proportional errors
- ❖ Absolute and relative errors

1.12 SELF-ASSESSMENT QUESTION AND EXERCISES

1. Define error by two deferent main term?
2. Give the examples for systematic and random errors?
3. What are instrumental errors?
4. Write about the proportional errors with example?
5. Define absolute error?
6. Write down the concept of relative error?

1.13 Further readings

1. Chatwal and Anand, Instrumental methods of chemical analysis, Himalaya publishing House New Delhi, 2000.
2. D.B.Hibbert and J.J. Gooding, Data Analysis for chemistry, Oxford University Press, 2006

Unit 2: DATA ANALYSIS

Structure

2.1 INTRODUCTION

Data analysis is the process of bringing order, structure and meaning to the mass of collected data. It is a messy, ambiguous, time-consuming, creative, and fascinating process. It does not proceed in a linear fashion; it is not neat. Qualitative data analysis is a search for general statements about relationships among categories of data. This unit 2 provides the information about accuracy and precision, mean and median, standard deviation, standard and normal error curves, significant figures and types of calculations involving significant figures

2.2 OBJECTIVES

After going through this unit, you will be able to

- ❖ To understating about accuracy and precision
- ❖ To know the basic concept of mean, median
- ❖ To know the basic of significant figures
- ❖ To define the basic calculation of significant figures.

2.3 ACCURACY AND PRECISION

Accuracy

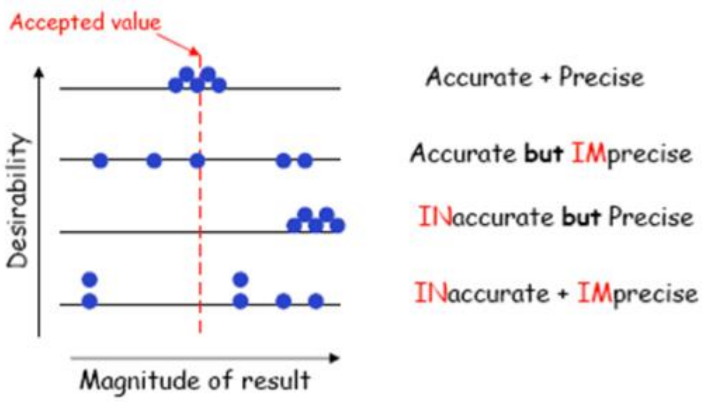
- ✓ Accuracy indicates the closeness of the measurement to the true or accepted value and is expressed by the error.
- ✓ Accuracy measures agreement between a result and the accepted value.
- ✓ Accuracy is often more difficult to determine because the true value is usually unknown. An accepted value must be used instead.
- ✓ Accuracy is expressed in terms of either absolute or relative error.

Precision

- ✓ Precision describes the agreement among several results obtained in the same way. Describes the reproducibility of measurements.
- ✓ Precision is readily determined by simply repeating the measurement on replicate samples.

- ✓ Precision of a set of replicate data may be expressed as standard deviation, variance, and coefficient of variation.
- ✓ d_i, deviation from mean, is how much x_i, the individual result, deviates from the mean.

$$d_i = |x_i - \bar{x}|$$



2.4 MEAN AND MEDIAN

Usually, the mean or the median is used as the central value for a set of replicate measurements. An analysis of the variation in the data allows us to estimate the uncertainty associated with the central value.

Mean

The mean, also called the arithmetic mean or the average, is obtained by dividing the sum of replicate measurements by the number of measurements in the set:

The symbol $\sum x_i$ means to add all of the values x_i for the replicates; x_i represents the individual values of x making up the set of N replicate

$$\bar{x} = \frac{\sum_{i=1}^{N} x_i}{N}$$

measurements.

Median

- ✓ The median is the middle value in a set of data that has been arranged in numerical order.
- ✓ The median is used advantageously when a set of data contain an outlier. An outlier is a result that differs significantly from others in the set.
- ✓ An outlier can have a significant effect on the mean of the set but has no effect on the median.

Example: Results from six replicate determinations of iron in aqueous samples of a standard solution containing 20.0 ppm iron(III). Note that the results range from a low of 19.4, 19.5, 19.6, 19.8, 20.1 and 20.3 ppm of iron. Calculate the mean and median.

Solution

$$Mean = \frac{19.4+19.5+19.6+19.8+20.1+20.3}{6} = 19.78$$

The average, or mean value, x, of the data is 19.78 ppm, which rounds to 19.8 ppm Fe.

Because the set contains an even number of measurements, the median is the average of the central pair:

$$Median = \frac{19.6 + 19.8}{2} = 19.7\ ppm\ Fe$$

2.5 STANDARD DEVIATION

The standard deviation of a given set of data is defined as the square root of the sum of the individual deviations squared, divided by the number of readings.

i.e S.D= $\sigma = \sqrt{d1^2 + d2^2 + \ldots\ldots + dn^2 / n} = \sqrt{\sum d^2 / n}$

Relative standard deviation

It is the further measure of precision. The RSD is defined as the ratio of the standard deviation and mean of the set of data. RSD is expressed in ppt.

RSD = s/ x X 1000 ppt.

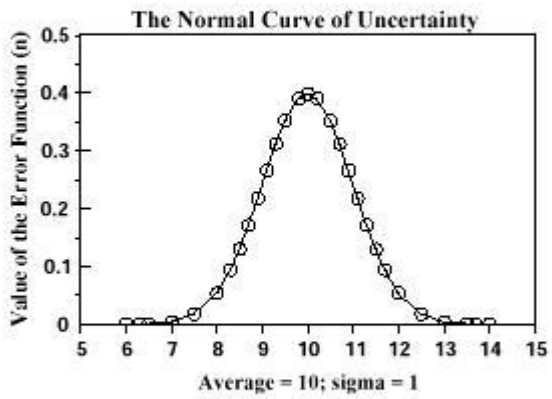
Variance

Variance is defined as mean square deviation.

i.e. Variance V = $\frac{\sum d^2}{n}$

2.6 STANDARD AND NORMAL ERROR CURVES

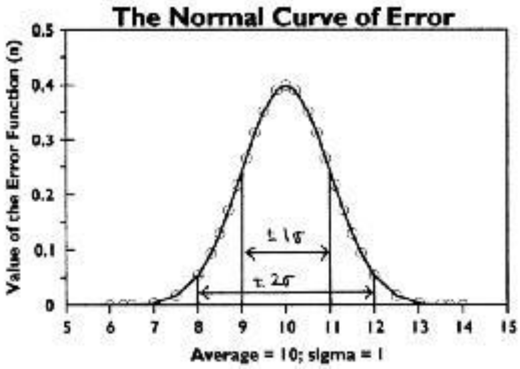
If you take a large set of data that has some amount of random error in it, the results will tend to follow the normal curve of uncertainty, often called the bell-shaped curve. The normal curve for the value 10 with standard deviation s = 1 is shown below.



NOTES

This curve shows up no matter what the data: if the errors are random (due to things like electrical noise and thermal motion) you will see this spread of data.

This curve has important implications for how we handle errors. The standard deviation, or "one-sigma" error, contains about 68% of the area of this curve



In the picture above, value 10+-1 is shown. About 68% of the area of the curve lies from 9 -> 11, the one sigma range. If we take another measurement, there is a ~68% chance that that measurement will fall between 9 and 11. This means that there is a ~68% chance that the actual value that we are trying to measure will also fall within this range. If you go out to 2s, 10+-2, about 95% of the curve lies between 8 -> 12, so there is an 95% chance that the true value falls in this range. A table of the sigma values and the chance that the true value falls in the given range for the value 10+-1 is shown below:

Average +- s	Range	Chance true value is in this area
10+-1*s	9 -> 11	~68.23%
10+-2*s	8 -> 12	~95.44%
10+-3*s	7 -> 13	~99.73%
10+-4*s	6 -> 14	~100%

As you can see, beyond 2s the chances that the true value lies in the +- 3 or 4 sigma range is very high.

We never have enough data to say for sure that the true value is within a certain range- there's always a tiny chance that it doesn't. (The normal curve never goes completely to zero.) In reality, we have to cut off the range somewhere: most scientists use the ~95% error range as a reasonable compromise.

Example: If you are given a set of data reported as 4.86 +- 0.09, what is the range that would give you a 68% confidence interval? What is the range for 95%?

Solution: A confidence interval of 68% corresponds to one standard deviation from the norm. Since the standard deviation is +- 0.09, there is a 68% chance that the true value falls within +-0.09 of the average value, or 4.77-> 4.95. The 95% confidence interval corresponds to two standard deviations, or 2*0.09 = +- 0.18. There is a 95% chance that the true value falls within 4.68-> 5.04.

2.7 SIGNIFICANT FIGURES

- Must review significant figures before considering errors and error analysis.
- Significant figures in a measurement include all certain digits plus the first uncertain digit.

1. For lined scales, estimate one digit smaller than the smallest division on the scale. For example:
    a) Graduated cylinders.
    b) Mohr pipets.
2. For electronic/digital readouts, all displayed digits are assumed significant, and the last digit is assumed to be only uncertain digit.

Rules to determine significant figures

- All non-zero digits are significant.
- Zeros used to locate a decimal point are never significant (i.e., zeros to the left of a nonzero digit).
- Zeros used to indicate the accuracy of the measurement are significant (i.e., zeros to the right of a nonzero digit in the presence of a decimal point).
- Trailing zeros (not indicating accuracy) may or may not be significant.
    i. If a decimal is present, they are significant.
    ii. If a decimal is not present, they are not significant.
    iii. It is always best to use scientific notation.

2.8 CALCULATIONS INVOLVING SIGNIFICANT FIGURES

a) Addition and subtraction

The absolute error (uncertainty) of the result is equal to the largest absolute uncertainty of the quantities added/subtracted. (Simply stated: "line 'em up and cut 'em off.").

For example,

1.256
0.2
<u>9.31245</u>
Sum = 10.96845 = 11.0

b) Rounding off convention

3.2456 ==> 3 significant figures ==> 3.25
3.2450 ==> 3 significant figures ==> 3.24
3.2449 ==> 3 significant figures ==> 3.24
3.151 ==> 2 significant figures ==> 3.2
3.150 ==> 2 significant figures ==> 3.2
3.149 ==> 2 significant figures ==> 3.1

Note that if the number is exactly halfway between numbers (i.e., like 3.150 in the example above), the last digit to be kept is rounded up if odd and rounded down if even.

NOTES

c) Multiplication/division

The usual method is that the quotient/product should have the same number of significant figures as the quantity multiplied/divided with the least number of significant figures.

By strict interpretation, the answer should have the same relative uncertainty as the factor with the largest relative uncertainty (i.e., the fewest significant digits).

For example:

(5,845)(98) = 572,810 (calculator)
= 570,000 (rounded to 2 significant figures)
= 573,000 (rounded to 3 significant figures)
Note that 98 almost has three significant figures (i.e., it's nearly 100).

d) Logarithms and Antilogarithms

1. A logarithm of "A" is a number "B" whose value is such that...
   $A = 10^B$ and $\log(A) = B$ (common log or log base 10)
   $A = e^B$ and $\ln(A) = B$ (natural log)
2. We will work exclusively with common logs.
3. Logarithms are composed of a mantissa and a character. For example...
   log(523) = 2.7185017 = 2.719 (with appropriate significant figures)
   2 ==> the character
   .7185017 ==> the mantissa
   Note that in scientific notation,
   $523 = 5.23 \times 10^{+2}$
   log(5.23) = 0.7185017 ==> the mantissa
   $\log(10^{+2}) = 2$ ==> the character
4. Significant figure rules for logs/antilogs:
   i. In a logarithm, keep as many significant figures in the mantissa as there are in the original number.
   ii. In an antilogarithm, report as many significant figures as are to the right of the decimal in the original number.

For example,
log(1,293) = 3.1115985 = 3.1116
$\text{antilog}(15.92) = 8.3176 \times 10^{+15} = 8.3 \times 10^{+15}$

e) Exact numbers have infinite significance

1. Exact numbers usually refer to a quantity that has a discrete amount (e.g., counted items).
2. Defined numbers are exact (e.g., 1 centimeter = 10 millimeters is a defined relationship).
3. π (3.1415926535....) is considered to be an exact number.

Check Your Progress – 2

1. What is accuracy?
2. Define mean?
3. What are type of calculations involving significant figures?
4. What is related standard deviation?
5. Define variation?

2.9 ANSWER TO CHECK YOUR PROGRESS QUESTION

1. Accuracy indicates the closeness of the measurement to the true or accepted value and is expressed by the error.
2. Accuracy measures agreement between a result and the accepted value.
3. Accuracy is often more difficult to determine because the true value is usually unknown. An accepted value must be used instead.
4. Accuracy is expressed in terms of either absolute or relative error.
5. The mean, also called the arithmetic mean or the average, is obtained by dividing the sum of replicate measurements by the number of measurements in the set:

$$\bar{x} = \frac{\sum_{i=1}^{N} x_i}{N}$$

6. Addition and subtraction, rounding off convention, multiplication/division, logarithms and antilogarithms and exact numbers have infinite significance
7. It is the further measure of precision. The RSD is defined as the ratio of the standard deviation and mean of the set of data. RSD is expressed in ppt.

   RSD = s/ x X 1000 ppt.

8. Variance is defined as mean square deviation.

$$\text{i.e. Variance V} = \frac{\sum d2}{n}$$

2.10 SUMMARY

In summary, unit 2 briefly discussed about the data analysis. More briefly it deals how to analysis an accuracy and precision, mean and median, standard deviation in data analysis and standard and normal error curves. Also provides the knowledge about significant figures and types of calculations involving significant figures.

2.11 KEY WORDS

- Accuracy and precision
- Mean and median
- Significant figures
- Standard deviation
- Average deviation

2.12 SELF-ASSESSMENT QUESTION AND EXERCISES

1. What is precision?
2. Results from six replicate determinations of iron in aqueous samples of a standard solution containing 20.0 ppm iron(III). Note that the results range from a low of 19.4, 19.5, 19.6, 19.8, 20.1 and 20.3 ppm of iron. Calculate the mean and median?
3. Write the rules to determine significant figures?
4. Write any two type of calculations involved significant figures?
5. If you are given a set of data reported as 4.86 +- 0.09, what is the range that would give you a 68% confidence interval? What is the range for 95%?
6. Define relative standard deviation and variance?

2.13 FURTHER READINGS

1. Chatwal and Anand, Instrumental methods of chemical analysis, Himalaya publishing House New Delhi, 2000.
2. D.B.Hibbert and J.J. Gooding, Data Analysis for chemistry, Oxford University Press, 2006

Unit 3: COMPARISON OF RESULTS

Structure

3.1 INTRODUCTION

Comparison of results is very important if a laboratory performs the same test using different methodologies or instruments, or performs the same test at multiple testing sites, the laboratory must have a system that twice a year evaluates and defines the relationship between test results using the different methodologies, instruments, or testing sites. The laboratory must have a system to identify and assess patient test results that appear inconsistent with the relevant criteria. Herein, unit 1 provide basic criteria for comparison of results such as confidence limit, correlation coefficient and regression analysis, comparison of methods and least squares method for deriving calibration graph

3.2 OBJECTIVES

After going through this unit, you will be able to

- To know the basic concept of confidence limit
- To understating the correlation coefficient and regression analysis
- To know the F-test, T-test and Q-test
- To know the basic of least squares method.

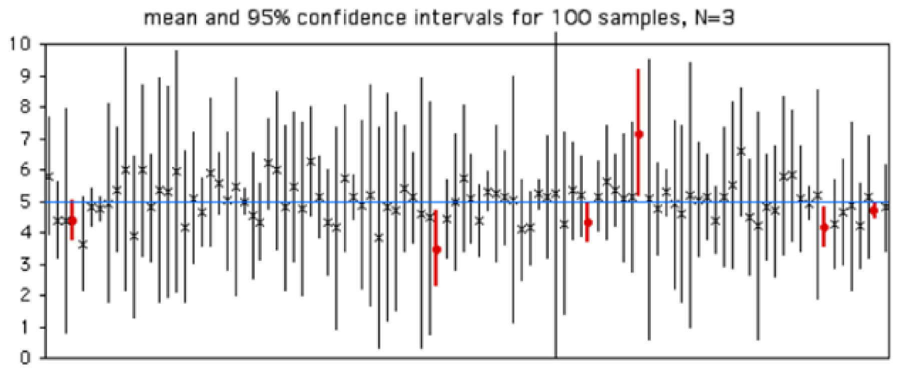
3.3 CONFIDENCE LIMIT

Confidence limits are the numbers at the upper and lower end of a confidence interval; for example, if your mean is 7.4 with confidence limits of 5.4 and 9.4, your confidence interval is 5.4 to 9.4. Most people use 95% confidence limits, although you could use other values. Setting 95% confidence limits means that if you took repeated random samples from a population and calculated the mean and confidence limits for each sample, the confidence interval for 95% of your samples would include the parametric mean.

Comparison of Results

NOTES

To illustrate this, here are the means and confidence intervals for 100 samples of 3 observations from a population with a parametric mean of 5. Of the 100 samples, 94 (shown with X for the mean and a thin line for the confidence interval) have the parametric mean within their 95% confidence interval, and 6 (shown with circles and thick lines) have the parametric mean outside the confidence interval.



3.4 CORRELATION COEFFICIENT AND REGRESSION ANALYSIS

Correlation and Regression are the two analysis based on multivariate distribution. A multivariate distribution is described as a distribution of multiple variables. Correlation is described as the analysis which lets us know the association or the absence of the relationship between two variables 'x' and 'y'. On the other end, Regression analysis, predicts the value of the dependent variable based on the known value of the independent variable, assuming that average mathematical relationship between two or more variables.

Correlation

- The term correlation is a combination of two words 'Co' (together) and relation (connection) between two quantities.
- Correlation is when, at the time of study of two variables, it is observed that a unit change in one variable is retaliated by an equivalent change in another variable, i.e. direct or indirect. Or else the variables are said to be uncorrelated when the movement in one variable does not amount to any movement in another variable in a specific direction.
- It is a statistical technique that represents the strength of the connection between pairs of variables.
- Correlation can be positive or negative.
- When the two variables move in the same direction, i.e. an increase in one variable will result in the corresponding increase in another variable and vice versa, then the variables are considered to be positively correlated. For instance: profit and investment.
- On the contrary, when the two variables move in different directions, in such a way that an increase in one variable will result in a decrease in another variable and vice versa, this situation is known as negative correlation. For instance: Price and demand of a product.

The measures of correlation are given as under:

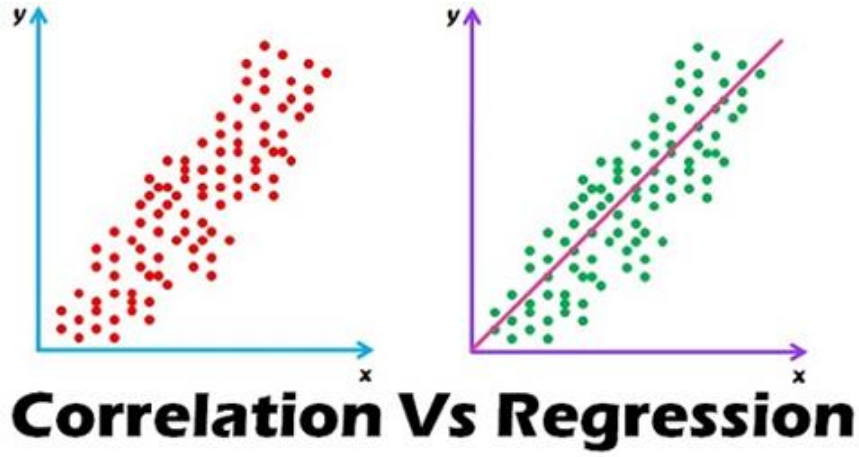
- Karl Pearson's Product-moment correlation coefficient
- Spearman's rank correlation coefficient
- Scatter diagram
- Coefficient of concurrent deviations

Regression

- A statistical technique for estimating the change in the metric dependent variable due to the change in one or more independent variables, based on the average mathematical relationship between two or more variables is known as regression.
- It plays a significant role in many human activities, as it is a powerful and flexible tool which used to forecast the past, present or future events on the basis of past or present events. For instance: On the basis of past records, a business's future profit can be estimated.
- In a simple linear regression, there are two variables x and y, wherein y depends on x or say influenced by x. Here y is called as dependent, or criterion variable and x is independent or predictor variable. The regression line of y on x is expressed as under:

$$y = a + bx$$

where, a = constant, b = regression coefficient, a and b are the two regression parameter.



BASIS FOR COMPARISON	CORRELATION	REGRESSION
Meaning	Correlation is a statistical measure which determines co-relationship or association of two variables.	Regression describes how an independent variable is numerically related to the dependent variable.
Usage	To represent linear relationship between two variables.	To fit a best line and estimate one variable on the basis of another variable.
Dependent and Independent variables	No difference	Both variables are different.
Indicates	Correlation coefficient indicates the extent to which two variables move together.	Regression indicates the impact of a unit change in the known variable (x) on the estimated variable (y).
Objective	To find a numerical value expressing the relationship between variables.	To estimate values of random variable on the basis of the values of fixed variable.

Table 3.3: Key differences between correlation and regression

3.5 COMPARISON OF METHODS

F-test

This test is comparing the precision of two sets of data. The procedure for the F- test is to calculate the F- value for the two sets of data using equation.

$$F= SA2/ SB2$$

Get the Fcri from the F-table which is calculated from a F-distribution, corresponding to the number of degrees freedom for the two sets of data.

T-test

The purpose of this test is to compare the mean from a small sample with the standard value and to test the difference between the means of two sets of data X1 and X2.

The following steps are involved in this test. For the given se of data, the ‘t’ value is first calculated using the equation

$$t=x-\mu\sqrt{N} / s$$

Where, μ is the standard or true value.

Then find out tcritical value from t-table at the desired confidence level, probability and number of degrees of freedom. Compare tcal value with that of tcri. If tcal <tcr, There is no significant difference between the mean and standard values or between mean results of two methods, etc. on the other hand, if tcal >tcri, there is significant difference between the two values.

NOTES

Q-test

In the rejection of a result, Q value of the result is calculated using the equation

$$Qexp = \frac{|\text{Questionable value} - \text{nearest value}|}{\text{Largest value} - \text{smallest value}}$$

The referring to the Qcritical value from the Q table, rejection of the data may do if Qexp exceeds the Qcri value.

Rejection criteria

In the data points if some of the data are very different, may be due to gross blunders or abnormal conditions during experiment or due to negligence of the observer then such data can be discarded straight away. This should be done only if the experimenter is very sure about the dubious nature of the data. There are many methods available for finding whether the particular data should be rejected or retained. The most commonly followed methods are

(i) Chauvenet's criterion
(ii) Use of Confidence intervals
(iii) 3σ limits.

3.6 LEAST SQUARES METHOD FOR DERIVING CALIBRATION GRAPH

The "least squares" method is a form of mathematical regression analysis used to determine the line of best fit for a set of data, providing a visual demonstration of the relationship between the data points.

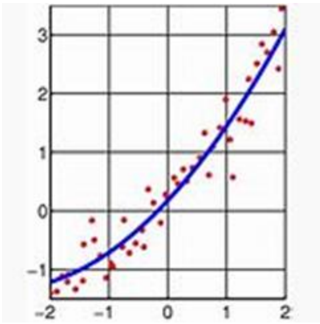
Each point of data represents the relationship between a known independent variable and an unknown dependent variable.

The least squares method provides the overall rationale for the placement of the line of best fit among the data points being studied. The most common application of this method, which is sometimes referred to as "linear" or "ordinary", aims to create a straight line that minimizes the sum of the squares of the errors that are generated by the results of the associated equations, such as the squared residuals resulting from differences in the observed value, and the value anticipated, based on that model.

This method of regression analysis begins with a set of data points to be plotted on an x- and y-axis graph. An analyst using the least squares method will generate a line of best fit that explains the potential relationship between independent and dependent variables.

In regression analysis, dependent variables are illustrated on the vertical y-axis, while independent variables are illustrated on the horizontal x-axis. These designations will form the equation for the line of best fit, which is determined from the least squares method.

In contrast to a linear problem, a non-linear least squares problem has no closed solution and is generally solved by iteration. The discovery of the least squares method is attributed to Carl Friedrich Gauss, who



discovered the method in 1795.

Check Your Progress – 3

1. What is confidence limit?
2. What is correlation coefficient?
3. What is regression analysis?
4. Define the F-test?
5. Write down the information of Rejection criteria?

3.7 ANSWER TO CHECK YOUR PROGRESS QUESTION

1. Confidence limits are the numbers at the upper and lower end of a confidence interval.
2. Correlation is described as the analysis which lets us know the association or the absence of the relationship between two variables 'x' and 'y'.
3. Regression analysis, predicts the value of the dependent variable based on the known value of the independent variable, assuming that average mathematical relationship between two or more variables.
4. This test is comparing the precision of two sets of data. The procedure for the F- test is to calculate the F- value for the two sets of data using equation.

$$F= SA2/ SB2$$

Get the Fcri from the F-table which is calculated from a F-distribution, corresponding to the number of degrees freedom for the two sets of data.

5. In the data points if some of the data are very different, may be due to gross blunders or abnormal conditions during experiment or due to negligence of the observer then such data can be discarded straight away. This should be done only if the experimenter is very sure about the dubious nature of the data. There are many methods available for finding whether the particular data should be rejected or retained. The most commonly followed methods are

(i) Chauvenet's criterion
(ii) Use of Confidence intervals
(iii) 3σ limits.

3.8 SUMMARY

In summary, unit 3 briefly discussed about the comparison of results. More briefly it deals about confidence limits and the basic understanding to the correlation coefficient and regression analysis and its comparisons. Additionally this unit deals the comparison methods such as F-test, T-test, Q-test and least squares method.

3.9 KEY WORDS

- ❖ Confidence limit
- ❖ Regression analysis
- ❖ Comparison methods
- ❖ Rejection of data
- ❖ Least square method

3.10 SELF-ASSESSMENT QUESTION AND EXERCISES

1. Define correlation and regression?
2. How to measure the correlation?
3. Write down the concept of F-test?
4. What is the common method used to find rejection?
5. Descript the main two different between the correlation coefficient and regression analysis?
6. Write down the concept of Q-test?

3.11 FURTHER READINGS

1. Chatwal and Anand, Instrumental methods of chemical analysis, Himalaya publishing House New Delhi, 2000.
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BLOCK-II
ELECTROANALYTICAL METHODS

Unit 4: ELECTRODICS

Structure

4.1 INTRODUCTION

The study of electrochemistry began in the 18th century, bloomed until the early 20th century, and then faded, owing to an excessive use of thermodynamic principles in analyzing the processes that take place at points in the system where the various parts form interfaces. Since about 1950 electrochemistry has undergone a change. The study of processes in solutions has been less stressed, but the study of the transfer of electrons between metals and solution has increased explosively. With this new emphasis electrochemistry is becoming a core science. In this unit 4 retains its original use of the term ‘electrodics’, a word that my spell checker is reluctant to accept and which even more reluctant to add to it. Electrodics is the electrochemistry of phenomena occurring at the surface of electrodes, particularly charge-transfer reactions. This unit briefly deals about some basic analytical methods such as ion selective electrodes, potentiometric methods, electrogravimetry and coulometric analysis

4.2 OBJECTIVES

After going through this unit, you will be able to

- ❖ To know the basic principle of electrodics
- ❖ To know the ion selective electrodes
- ❖ To determine the potentiometric and electrogravimetry analysis

❖ To know the different methods in electrodics.

4.3 ION SELECTIVE ELECTRODES

An ion-selective or specific ion electrodes (ISE or SIE) is a transducer or a sensor that converts the activity of a specific ion dissolved in a electrolyte into a electrical potential. Similarly, many types of membrane based electrodes have been developed in which the membrane potential is selective toward a given ion or ions, in the same way as the potential of glass electrode is selective towards hydrogen ions. An ion selective electrodes are widely used as a powerful tool in electroanalytical chemistry including biochemical and biophysical researches for rapid, sensitive, non-destructive and precise determination of many ions in a great variety of media; some of these electrodes are given below:

1. Glass membrane electrodes
2. Precipitate electrodes
3. Solid-state electrodes
4. Liquid-liquid electrode
5. Enzyme electrodes

4.3.1 Instrumentation

The glass electrode has high resistance, therefore, one electronic instrument called pH meter is generally used for determining the pH of the solution. There are two types of pH meters.

(i) Potentiometric type
(ii) Direct reading type

(i) Potentiometric type

In this instrument, the glass electrode is incorporated in an ordinary potentiometric circuit and the off-balance currents are amplified electronically so that a milliammeter can be used to detect the balance point. The electronic amplifier in this manner acts as a null point indicator. In this type a slide wire is adjusted until no meter deflection is obtained. This wire is calibrated directly in pH units and also in millivolts. The schematic diagram of electronic circuit is shown in Figure 4.3.1.

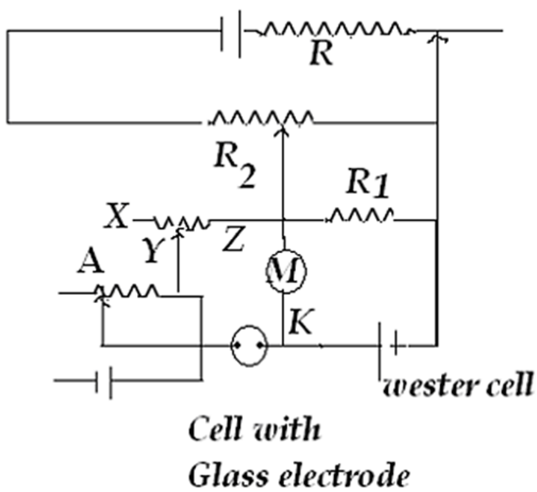


Figure 4.3.1: Schematic diagram of electronic circuit

XYZ is a potentiometer which is directly calibrated in pH units. The voltage corresponding to one division of the potentiometer should be adjusted according to the temperature of the solution. For making the temperature adjustments, the potentiometer *XYZ* is shunted with the resistance *R2*. The current pass through the potentiometer is adjusted with resistance *R1* which is firstly calibrated against the Weston cell. The key *k1* is allowed to remain in position 1 by making the adjustment with the variable resistance *R*. *A* is a potentiometer which is connected in series with the main potentiometer *XYZ* and acts to correct for variations in the constant *k* according to the equation:

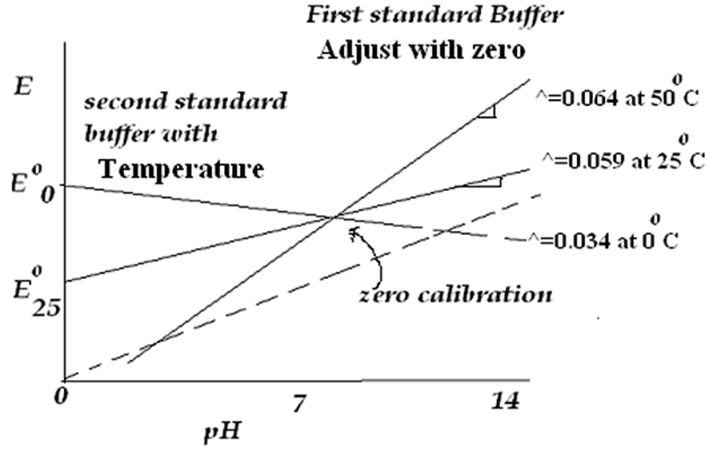
$$E = k + 0.0591\ pH$$

When the pH meter is in operation, the E.M.F developed by the glass and reference cell is opposed by E.M.F obtained from *XYZ*. Any off balance is observed as deflection on the volt meter and the main potentiometer is then adjusted until the deflection is zero. Now for measuring unknown pH the instrument is first calibrated with a buffer solution of known pH and then for unknown pH and the position of *Y* is changed until balance is obtained in a position when k is in position 2.

(ii) Direct reading type

The sensitivity of the meter (scale division/mV) is adjustable by a temperature control knob to allow for a change in the 0.059 factor with temperature. To read absolute mV rather than pH, the temperature control is disconnected entirely. The zero calibration knob changes the zero setting of the meter by introducing a bias voltage into the meter circuit so that the meter reading will correspond to the pH of the standard buffer.

These instruments are often constructed so that the true zero of the voltmeter is at pH 7 on the scale. In use, the pH meter should be first standardized with a pH 7 buffer, adjusting the zero calibration to give the correct reading. Then a second buffer, pH 4 or 10 is used and pH is adjusted to give the correct reading with the temperature control. This procedure establishes the correct linear relationship between E (mV) and



pH (Figure 4.3.2).

Figure 4.3.2: Linear relationship between E (mV) and pH

At least two points are required to establish the line, although for rough measurements one point and theoretical slope of 0.059 may be adequate for calibration.

4.3.2 Applications

The dynamic properties of ion-selective electrodes are of great practical importance for different analysis in various fields. Some examples are mentioned for fellow:

Agriculture

- Soil samples - determination of calcium, nitrates, sodium, potassium, boron, ammonium and halogenides
- Fodder - measurements of nitrogen after Kjeldahlization, calcium, halogenides, sodium, potassium, nitrates, to ensure quality products
- Plant tissues - nitrates, halogenides, cyanides, calcium, sodium and potassium can be measured currently
- Fertilizers - determination of potassium, calcium, nitrogen and nitrates in fertilizers

Food industry

- meat and fish - nitrite and nitrate measurements in meat and fish processing, checking the fluoride level in fish protein to state the toxin concentration
- milk and dairy products - monitoring of fluoride concentrations for checking some toxins
- drinks and juices - determination of the concentration of chlorides, fluorides and carbonates
- alcoholic drinks, beer, wine - determining of potassium, sodium, carbonate, fluoride, and/or bromide levels

Medicine

- Blood, serum - measurement of potassium, calcium, sodium, fluorides in samples
- Urine - easy determination of fluorides, ammonium and calcium

Medical products

- Determination of fluorides in vitamines and tooth pastes, halogenides, copper, nitrates, or, if need be, calcium can be determined in a number of medicaments

Photochemistry

- Bromide, nitrate and silver concentration measurements

Paper industry

- Sodium, calcium, silver, sulphide and chloride concentration measurements

Metallurgy and electroplating baths

- Concentration measurements of copper, cadmium, free and total cyanides, fluorides, fluoroborates, nitrates and ammonium

Power engineering

- Calcium and chloride concentration can be easily measured in boiler and exchanger waters

Water systems

- Natural water - calcium, potassium, sodium, silver, lead, cadmium, halogenide, ammonium, sulphide and carbonate ions are currently determined
- Drinking water - fluorides and nitrates are most frequently measured substances
- Sea water - determination of halogenide, nitrate, potassium and sodium ions
- Waste water - concentration measurements of copper, silver, cyanides and ammonium, and, if need be, of nitrogen after Kjeldahlization

Determination of acidity

- In all the mentioned fields and branches, where the ion selective electrodes are used, the glass pH-electrode is also used.
- The determination of acidity is, in most cases, an essential condition for the qualification of the samples under question.

Education and Research

- At present, the electrodes are an important implement for numerous analytic and instrumental subjects. They help to observe effects and processes concerning the kinetics, equilibrium states and to determine activity coefficients, solubilities and ionizations
- The electrode are used in following fields and branches: food industry, agriculture, agronomy, medicine, chemistry, physics, biology, stomatology, enviroment protection, etc.

4.4 POTENTIOMETRIC METHODS

4.4.1 Principle

Potentiometric methods include two major types of measurements

(i). Direct measurement of an electrode potential from which the concentration of an active ion may be found.

(ii). Changes in the E.M.F of an electrolytic cell brought about by the addition of a titrant. These methods are based on the quantitative relationship of the E.M.F of a cell as given by the following equation.

$$E_{cell} = E_{reference} + E_{indicator} + E_{junction}$$

The reference electrodes are expected to assume a potential which is independent of the composition of the solution. Moreover, the junction potential is assumed to remain almost constant. Under these conditions, the indicator electrode can give information about the nature or concentration of substances capable of exchanging electrons.

In the potentiometric titration, the course of titration reaction is followed by measuring the concentration of one or more of the species potentiometrically. The titration beaker becomes one of half-cells along with a convenient reference electrode for the other half-cell. It is important to distinguish between the titration reaction and cell reaction. The reactants and products of the titration are all in same half-cell and the titration reaction is always at equilibrium. The cell reaction is normally not at equilibrium because it is not allowed to take place except for brief periods while balancing the potentiometer. The changes in the electrode potential upon the addition of the titrant are noted against the volume of the titrant added. At the end point the rate of change of potential is high. The end point is found by plotting a curve of potential versus the volume of the titrant. The potentiometric end point has been applied to all types of chemical reactions. It can be used with colored or opaque solutions that mask observation of ordinary indicator changes.

4.4.2 Instrumentation

Three types of instruments are used for the measurement of potentials.

i. Non-electronic instruments
ii. Electronic instruments
iii. Automatic instruments

(i). Non-electronic instruments

A potentiometer for titrations can be made from simple components as shown in Figure 4.4.1.

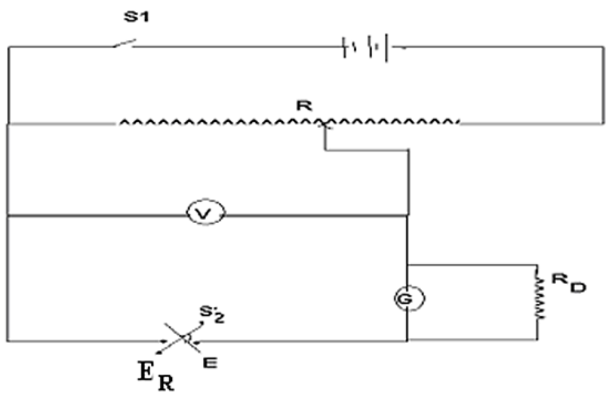


Figure 4.4.1: Set up for potentiometer

In this apparatus battery which supplies working current and consists of two dry cells in the series. R is a variable resistor and V is a voltmeter. A galvanometer, G, of intermediate sensitivity is critically damped by the resistor RD. A reversing switch S2 helps in connecting the reference and indicator electrodes in either polarity. The instrument can be operated by dipping the electrodes in the sample solution, closing S1 and adjusting R

until there is no deflection in the galvanometer. Finally, the voltmeter reading is recorded. If balance point is not obtained, then S2 is reversed and the process is repeated after each addition of reagent.

(ii). Electronic instruments

These instruments have many advantages over non-electronic instruments. Due to high resistance of the glass electrode, it cannot be used with a simple potentiometer. A electronic amplifier for use with a glass electrode is called a pH meter. These pH meters with glass electrodes and electronic voltmeters for pH measurements and potentiometric titrations are commercially and available. A schematic diagram of a circuit for vacuum triode null point detector is shown in Figure 4.4.2.

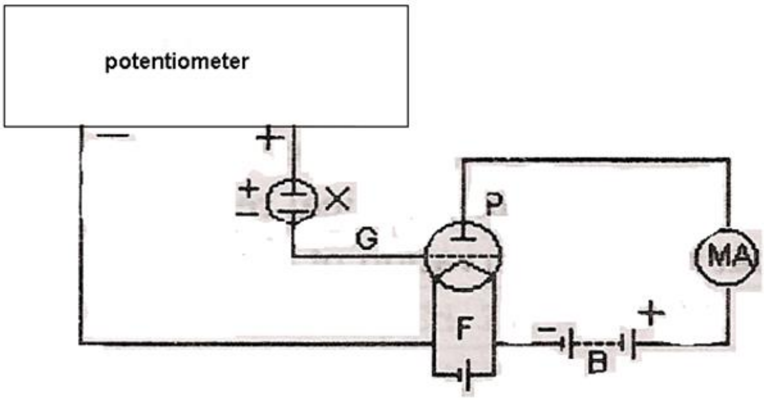


Figure 4.4.2: Set up for Electronic potentiometer

The vacuum tube or triode consists of a cathode F, a plate P and a grid G. When the cathode F is heated by a source of low voltage, the electrons are thrown out. These electrons are attracted towards plate P by virtue of positive charge on P produced by its being connected to the positive pole of the battery B. The small current passing through the external plate is indicated by the milliammeter MA. The flow of electrons is repelled back from the cathode to the plate by impressing a negative charge on the grid G. This reduces the plate current indicated by milliammeter. The negative terminal of the cell X is joined to the grid, so that any unbalance between the potential of X and potentiometer will cause a deflection in millimeter. Adjusting the potentiometer to the position corresponding to no charge on the grid gives the potential drop in X then restores the balance.

(iii). Automatic instruments

A manual potentiometric titration is a time consuming and tedious job. Automatic instrument for performing and recording the titration curve provides a logical solution to the problem. In the delivery unit a short length of the flexible tubing is shut in some way, with no current passing through the solenoid. After setting the instrument and reading the burette level, a switch is pressed to start the titration. The solenoid is energized, the pressure on the tubing is released, and then the titrant is allowed to flow through the delivery tip. The titration proceeds at a fast rate until a predetermined distance from the end point, when the anticipation control automatically shows the delivery of the titrant. At the end point the

delivery is stopped. A schematic circuit diagram of an automatic titrator is shown in Figure 4.4.3.

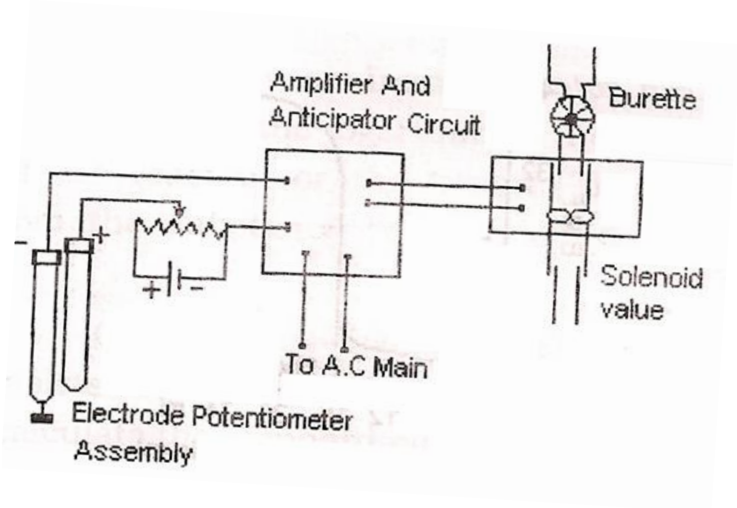


Figure 4.4.3: Set up for Autotitrator

The control unit has calibrated potentiometer, a null-sensing amplifier and an anticipator circuit. To operate, the potentiometer is set at the potential expected at the end point, the electrode assembly is immersed in the sample solution and the operating switch is pressed. The difference signal arising between the cell, E.M.F and the present voltage on the potentiometer is amplified. As the end point is approached, the difference signal diminishes. When the two signals are matched, the delivery of the titrant is stopped.

The automatic titrators are no more accurate than manually performed titrations. Thus, they are most useful in those situations where speed is the predominant factor.

4.4.3 Applications

Potentiometric titrations may be applied to a variety of systems involving,

(i). Reduction-Oxidation (Redox) titrations
(ii). Precipitation titrations
(iii). Complexation equilibrium reactions
(iv). Acid-Base titrations

4.5 ELECTROGRAVIMETRY

4.5.1 Principle

In electrogravimetry, the passage of electric current causes the deposition of a metal upon an already weighed inter (working) electrode. After the deposition, the electrode is again weighed to find out the weight of the metal deposited.

The products of electrolysis exert a back E.M.F. This reduces the actual E.M.F of the cell. Thus, the electrolysis of an electrolyte is possible only when this back E.M.F is overcome. For example, two platinum electrodes are placed in a dilute solution of copper sulfate. No appreciable current will flow through the system, until some minimum potential is

applied. The current will increase as the applied potential increases after this minimum potential. The applied voltage, which is just sufficient to overcome the back E.M.F due to polarization and also to bring about the electrolysis of an electrolyte without any hindrance, is known as the decomposition potential.

The decomposition potential, Ed is composed of various potentials and given by the following equation.

$$E_a\,(min) = E_d + E_B + E_s + E_V$$

Where, E_a= applied potential, E_d=decomposition potential; E_B= theoretical counter or back potential, E_s= system potential or resistance potential and E_v = over voltage.

The system potential is also known as resistance potential and is defined, as the potential required overcoming the resistance of the system. This can be calculated by using Ohm's law i.e. $E=IR$. An increase of the applied potential above the decomposition potential causes an increase in E_s because the amount of current flowing through the system increases with increases of potential in this region.

When a potential is applied to the electrolyte cell, the source of potential compels electrons to move through the connector to one of the electrodes and tends to pull electrons from the other electrode. The positively charged electrode (anode) accept electrons and the negatively charged electrode (cathode) gives electrons. As a result, the positively charged ions in the solution (Cu^{2+}, H^+ if the solution in $CuSO_4$) are attached and moved towards the negative electrode. The negative ions in the solution (SO_4^{2-} and OH^-) move towards the positive electrode. On passing current copper ions are deposited on the cathode as copper atoms, because copper is discharged at lower potentials than the hydrogen ion. At the anode, hydroxyl ions are converted to oxygen molecules and hydrogen ions becomes a copper-copper ion half-cell and the anode becomes an oxygen half-cell. If the source of potential is removed, the electrolytic cell will behave like a galvanic cell. The potential that would be produced if the counter potential or back potential, the theoretical counter potential EB is given by

$$E_B = E_{anode} - E_{cathode}$$

Following factors also affect the over voltage. Such as current density, hydrogen ion concentration, temperature, impurities and pressure.

4.5.2 Instrumentation

The apparatus required for an analytical deposition consist of a suitable cell and direct- current power supply

(a). Cell

A typical cell is shown in figure 4.5.1.

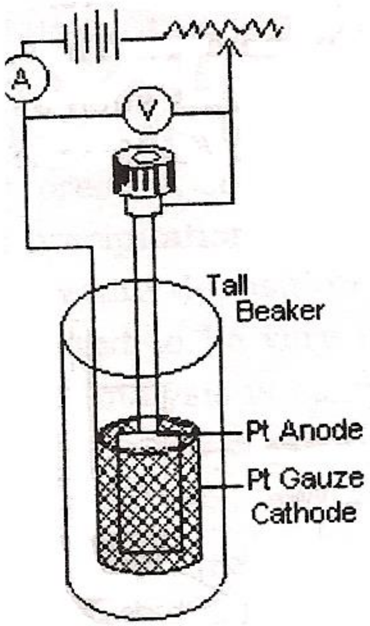


Figure 4.5.1: Cell for Electrodeposition of metals

Generally a tall beaker is used. Anode is made to rotate with an electric motor. Mechanical stirring will minimize concentration polarization. The voltage is measured by voltmeter V and current is measured by ammeter A.

(b). Electrodes

Generally the electrodes are made from platinum. However, electrodes made from copper, brass and other materials are sometimes used. The various advantages of using platinum electrodes over other metal electrodes are as follows, 1. Platinum electrodes are relatively non-reactive and 2. Platinum electrodes can be ignited to remove any grease, organic matter of gases that could have changed the physical properties of the deposit.

(c). Power supplies:

The DC power supply may consist of a storage battery, a generator or an alternating current rectifier. The purpose of using a rheostat is to control the applied potential. The ammeter and voltmeter are provided to indicate the approximate current and applied voltage. The apparatus required for a controlled potential electrolysis is shown in Figure. 4.5.2.

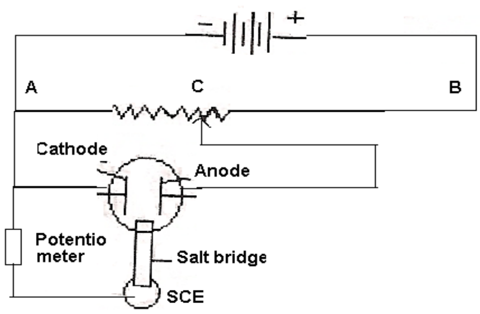


Figure 4.5.2: Set up for Electrogravimetry

4.5.3 Applications

- The equipment required for constant current electrolysis is simple as compared to that of constant cathode-potential methods. The common elements (As, Cu, Zn, Cd, Sn, Co, Ni etc.,) are listed and which have been determined by electrogravimetric procedures for which control over the cathode potential is not required.

- This method has been used successfully for analyzing solutions containing a mixture of the metallic elements used for successfully the determination of copper, bismuth, lead and tin.

4.6 COULOMETRIC ANALYSIS

4.6.1 Principle

Coulometric methods of analysis are based on the exact measurement of quantity of electricity that passes through a solution during the occurrence of an electrical reaction. The principle of coulometric method of analysis is based on Faraday's law. The extent of chemical reaction at an electrode is directly proportional to the quantity of electricity passing through the electrode. Thus 96,500 coulombs are equivalent to one gram equivalent of any element under identical conditions of 100% efficiency of the reaction. The fundamental requirement of coulometric method of analysis is that it can be applied only in such cases where the electrolytic reaction proceeds with 100% efficiency. The substance of interest may be oxidized or reduced at one of the electrodes. This analysis is called primary coulometric analysis and in this analysis, the substance to be estimated reacts at an electrode which is maintained at a constant potential with respect to the solution. The substance of interest may also react quantitatively in solution with a single product of electrolysis and the method is called secondary coulometric method. In this analysis, one of the products of electrolysis reacts with the substance to be estimated and the process is often carried out under constant current conditions and an end point indicator is required. It is well evident of that if in an experiment, Q coulombs of electricity have been expended, the weight of the element W deposited will be, $W=AQ/n\text{-}96500$.where A = atomic weight, n = valence of the element. N can be obtained from published data for the calculation of Q or W, any one of these quantities can be calculated provided other values are known from the experiment. Thus coulometric methods are those analytical methods which are based upon the measurement of a quantity of electricity and the application of the above equation.

Current measuring device

Coulometer is a device for measuring quantity of electricity by determining the amount of chemical change brought about by the current. Various type of coulometer such as oxygen hydrogen coulometer, silver culometer etc are in used.

4.6.2 Types of coulometric analysis

There are two different types of culometric analysis are available; (i) Constant current culometry analysis and (ii) Controlled potential culometric analysis.

(i). Constant current culometry analysis

In this technique, solution of the substance to be determined is electrolyzed at constant current. The completeness of the reaction can be detected by a visual indicator in the solution or by amperometric, potentiometric or spectrophotometric methods and circuit is then opened. The amount of electricity passed is derived from the product, current (Amp) x time (Sec). Constant current technique can be further divided into two parts.

(a). Primary constant current coulometry

(b). Secondary constant current coulometry or coulometric titrations

(ii). Controlled potential culometric analysis

In this technique the substance being determined reacts with 100% efficiency at a working electrode, the potential of which is controlled. The completion of the reaction can be obtained by the current decreasing particularly to zero.

4.6.3 Applications.

- Culometric analysis has been successfully applied to the estimation of halides at silver anode, mustered gas by titration with electrically generated bromine.
- The culometric titration of weak acids in nonaqueious solution have been carried out using silver and Pt electrodes and a supporting electrolyte of 0.2 M in sodium perchlorate.
- The culometric methods has been applied to the deposition of metals at a mercury cathode.

Check Your Progress – 4

1. What is ion selective electrode?.
2. Write the any five fields of applications of ISE?
3. What are types of instruments used to measure the potentials?
4. Write down any one application of electrogravimetry?
5. Mention the method which is used to deposite the metals at a mercury cathode?

4.7 ANSWER TO CHECK YOUR PROGRESS QUESTION

1. An ion-selective or specific ion electrodes (ISE or SIE) is a transducer or a sensor that converts the activity of a specific ion dissolved in a electrolyte into a electrical potential.

2. a. Agriculture, b. Food industry, c. Medicine, d. Medical products and e. Photochemistry.

3. Three types of instruments are used for the measurement of potentials.

   (i). Non-electronic instruments

   (ii). Electronic instruments

   (iii). Automatic instruments

4. Electrogravimetric has been used successfully for analyzing solutions containing a mixture of the metallic elements used for successfully the determination of copper, bismuth, lead and tin.
5. The culometric methods has been applied to the deposition of metals at a mercury cathode.

4.8 SUMMARY

In this unit 4, discussed about the detailed introduction, explanation and applications of electrodics. Briefly, this unit summarized the principles, instrumental set up and its applications for fallowing methods such as ion selective electrodes, potentiometric methods, electrogravimetry analysis and coulometric analysis.

4.9 KEY WORDS

- ❖ Ion selective electrodes
- ❖ Potentiometer
- ❖ Electronic potentiometer
- ❖ Electrogravimetry
- ❖ Coulometry

4.10 SELF-ASSESSMENT QUESTION AND EXERCISES

1. What is specific ion electrode?
2. Write the types of pH meters?
3. How did ISE used in water system, education and research?
4. Write the principle of potentiometric methods?
5. What is electrogravimetry analysis?
6. Define the types of coulometric analysis?

4.11 FURTHER READINGS

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2. Bard, Allen J., and Cynthia G. Zoski, eds. (2010) Electroanalytical chemistry. CRC press, London, New York.
3. Monk, Paul MS. (2008) Fundamentals of electroanalytical chemistry. Vol. 29. John Wiley & Sons.

Unit 5: POLAROGRAPHY

Structure

5.1 INTRODUCTION

J Heyrovsky's invention of polarography in which a dropping mercury electrode is used in the electrochemical cell revolutionised electroanalysis, study of electrode kinetics and adsorption studies on mercury electrodes. Polarography has also directly contributed to the development of new and innovative ideas in electrochemical techniques, instrumentation and applications. The question often posed is whether polarography has already seen the glorious days and whether its perceived decline is irreversible. It is true that the utility of polarography as an analytical tool in recent times is declining. This is true even of other electroanalytical techniques as methods of choice for chemical analysis. This is mostly due to advances in spectroscopic techniques. However, the contribution of polarography is not limited to electoanalysis alone nor is it limited to mercury as electrode material. The increasingly popular voltammetric techniques applied to solid electrodes were all enriched by the polarographic techniques. Thus, ever since its discovery in 1920s, its course had been running parallel to many other areas of electrochemistry and as such it is truly a trail blazing technique.

5.2 OBJECTIVES

After going through this unit, you will be able to

- ❖ To know the basic principles of polarography
- ❖ To find out the types of polarography
- ❖ To understanding about dropping mercury electrode
- ❖ To know the advantages of dropping mercury electrode.

5.3 PRINCIPLES OF POLAROGRAPHY

Polarography an electrochemical method for qualitative analysis, quantitative analysis, and the study of kinetics in chemical processes. Polarography was first proposed by J. Hey-rovsky and later developed by A. N. Frumkin and other scientists. It is based on the interpretation of current-voltage curves, called polarograms, which are produced during electrolysis of the solutions under study and express the dependence of current intensity on the direct current (DC) potential E_{dir} applied to the electrolytic cell. To produce polarograms, which are recorded by means of polarographs, the solution is placed in a cell with a reference microelectrode and an indicating electrode; a dropping mercury electrode (DME) with a renewable surface is most often used as the reference microelectrode. The electrode reaction occurring at the reference microelectrode produces neither significant chemical changes in the solution nor any marked difference in potentials because the reference microelectrode is always considerably smaller than the indicating electrode.

5.4 INSTRUMENTATION

A simple cell and electrical circuit of a polarograph are shown in Figure 5.4.1.

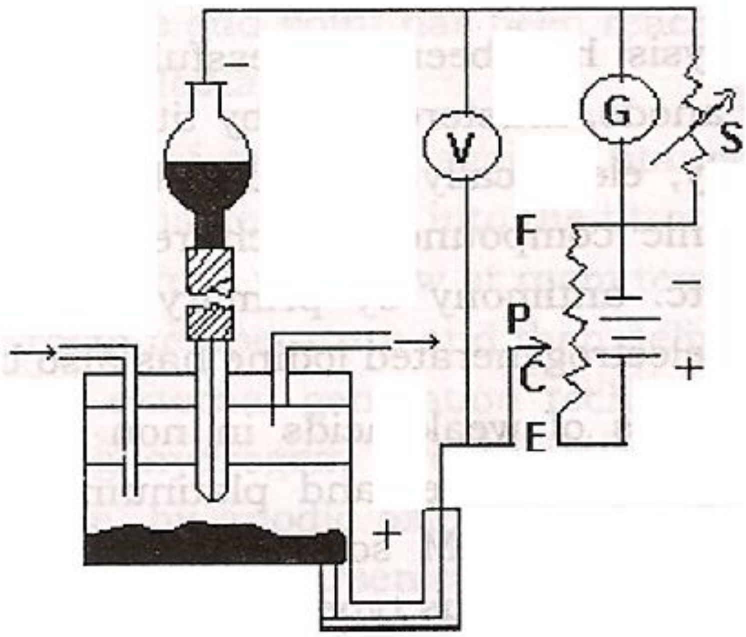
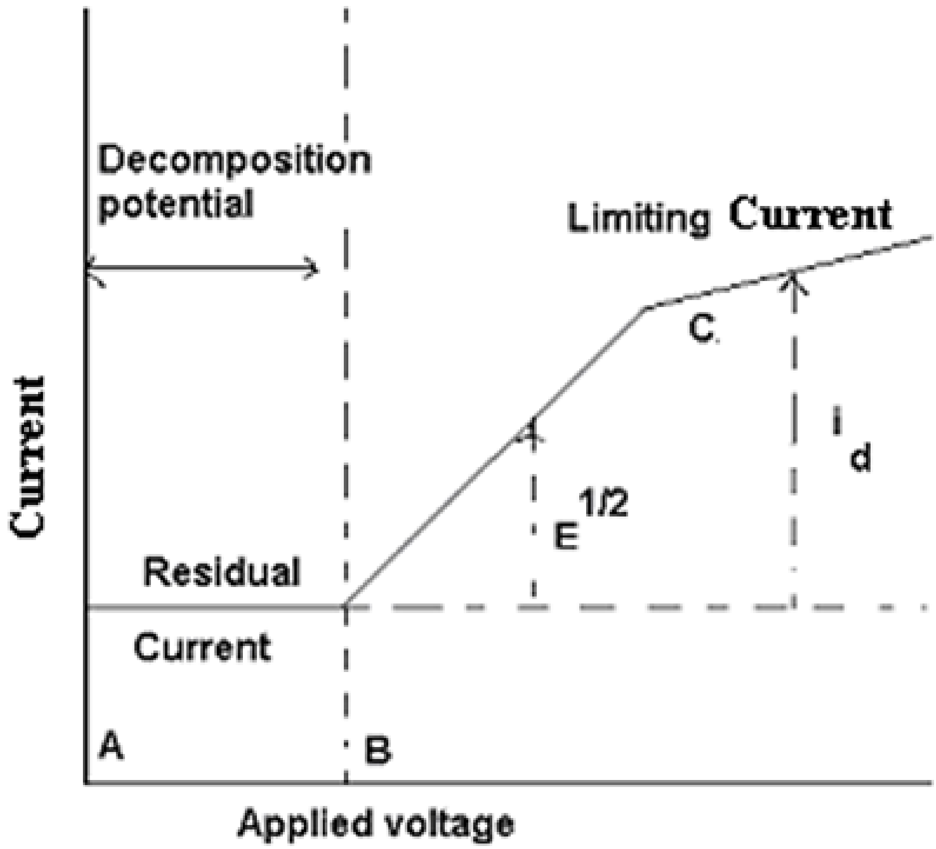


Figure 5.5.1: Setup for polarography

In this apparatus, there is dropping mercury electrodes which consist of mercury reservoir from which mercury drops down as small drops through a capillary. This acts as a cathode and is generally known as indicator or microelectrode. The anode consists of mercury pool at the bottom of the reservoir. Since its area is larger, it is not polarized. Both cathode and anode are connected across the appropriate ends of a battery. Adjusting the sliding contact along the E.M.F up to three volts may be readily applied to the cell. G is a galvanometer, which measures the current strength and S is a shunt for adjusting the sensitivity of the galvanometer.

The reservoir is provided for blowing nitrogen gas through the sample, which removes dissolved oxygen from the sample. The positively charged ions present in the solution will be attracted to the dropping mercury electrode by an electrical force and by a diffusive force resulting from the concentration gradient formed at the surface of the electrode. Thus, the total current flowing through the cell may be regarded as the sum of electrical and diffusive forces. When the applied voltage is increased and the current is recorded, a graph will be obtained which is shown in Figure



5.4.2.

Figure 5.4.2: Graph of polarogram

It can be seen from the figure that from A to B, a small current flows. This is known as residual current and is carried by the supporting electrolyte and impurities present in the sample. At point B, the potential of the electrode becomes equal to the decomposition potential of the positive ion. The current then increases along the curve BC. At point C current no longer increases linearly with applied voltage but reaches a steady limiting value at point D. After this no increase in current is observed at higher cathode potentials. Thus, the current corresponding to the curve CD is known as limiting current is called diffusion current and is generally denoted by I_d.

Factors affecting the limiting current:

1. Residual current
2. Migration current

3. Diffusion current
4. Kinetic current

5.5 TYPES OF POLAROGRAPHY

Various types of polarography are evaluated according to their sensitivity (the minimum concentration that can be determined) and resolution (the permissible ratio of concentrations of the supporting component to the component being determined) and depend on the shape and rate of change of the polarizing voltage.

(i) Direct-current (classical) polarography
(ii) High-frequency polarography
(iii) Pulse polarography
(iv) Oscillographic polarography

5.5.1 Direct-current (classical) polarography

In direct-current (classical) polarography, which is based on the dependence of electrolytic current I_e on the slowly varying polarizing E_{dir}, I_e is proportional to the number of electrons n that take part in the reaction. The sensitivity in determining reversibly reactive substances is 10^{-5} mole per litter *(M)*, and resolution is about 10. In alternating-current polarography, based on the dependence on E_{dir} of the alternating current I_{alt} that arises upon superimposition of various forms of a voltage E_{alt} ((low-amplitude rectangular, trapezoidal, and sinusoidal), I_{alt} is proportional ton^2. The high sensitivity of alternating-current polarography (10^{-7} M) results from the possibility of separating the effective signal I_{alt} from I_c, and its high resolution (up to several thousand) results from the bell shape of the polarogram (the ordinate rapidly tends toward zero upon a deviation of E_{dir} from peak potential) and by the possibility of determining reversibly reactive substances in the presence of components with irreversible reactivity (sensitivity in determining the latter is low).

5.5.2 High-frequency polarography

High-frequency polarography involves the superimposition of E_{dir} and a high-frequency E modulated by a low-frequency E. In this case I_{mf}, the component of the current for the modulated frequency, depends on E_{dir} and is proportional to n^3. The difference in variation between I_{mf} and I_c upon application of a high frequency is used to separate the effective signal I_{mf} from I_c. High-frequency polarography makes it possible to determine the rate constant of fast reactions.

5.5.3 Pulse polarography

Pulse polarography is based on the measurement of the current I_p, which arises upon application of an 0.04-sec voltage pulse at the moment when the surface of the mercury drop is maximal. The current I_p is separated from I_c by measuring I_p at the moment of damping of I_c. Pulse polarography has a sensitivity of $1–5 \times 10^{-8}$ M and resolution $\sim 5 \times 10^3$.

5.5.4 Oscillographic polarography

Oscillographic polarography is based on measurement of the dependence of *I*, on the rapidly varying E_{dir} (0.1–100 volts per sec). The polarograms produced in oscillographic polarography, which are recorded by means of a cathode-ray tube, have a distinct maximum.

In this type of polarography I_e is proportional to $n^{2/3}$, sensitivity is 10-6 M, and resolution is – 400. In addition to the DME, stationary mercury and solid electrodes are also used in polarography.

5.6 APPLICATIONS OF POLAROGRAPHY

Polarography is very widely used in monitoring the production of especially pure substances; in metallurgy, geology, and pharmacology; in the preparation of organic compounds and polymers; in medicine (for early diagnosis of diseases and for determining the presence of oxygen and trace elements in tissue and in products of vital activity); and in studying the mechanism of electrode reactions.

5.7 DROPPING MERCURY ELECTRODE AND ITS APPLICATIONS

A dropping mercury electrode (DME), in which drops are formed and fall off repeatedly during a potential scan, being replaced by a "fresh" electrode every second or so (Figure 5.7.1). The optimum interval between drops for most analyses is between 2 and 5 s. The toxicity of mercury has lead to a limited use these days, though it still is a very useful surface in methods that involve the preconcentration of a metallic analyte prior to potential scan, such as is done in anodic stripping voltammetry (ASV). Many practitioners now make use of mercury films formed on the surface of solid electrodes rather than the pure metal. Under these conditions, the small volume of the film allows analyte to concentrate at large values, with rapid diffusion times.

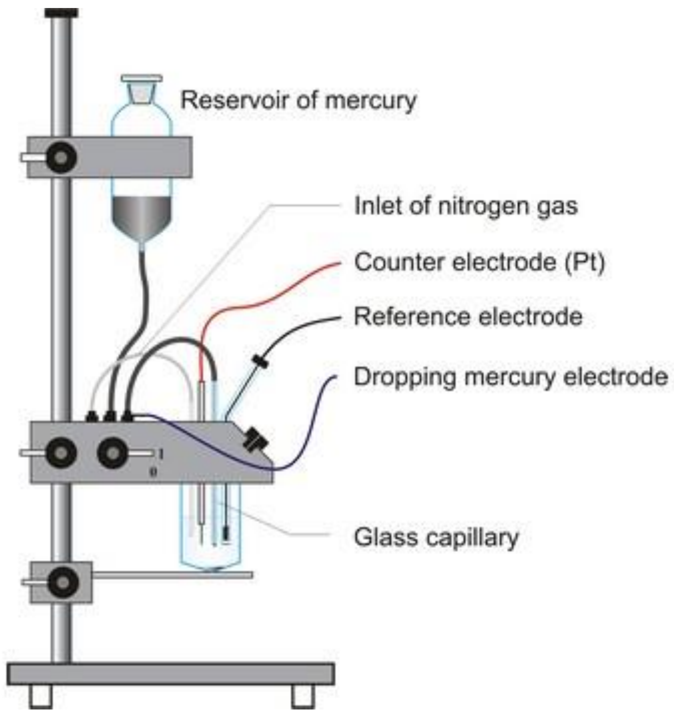


Figure 5.7.1: Image of dropping mercury electrode

5.7.1 Advantages of DME

The advantages of dropping mercury electrode are as follows:

- Mercury form amalgam with most metals.
- Mercury has a high hydrogen overvoltage.
- It provides a smooth, fresh surface for the reaction.
- Each drop remains unaffected and does not become contaminated by the deposited metal.
- Diffusion equilibrium is readily established at mercury-solution interface.
- The constant renewal of the electrode surface, exposed to the test solution, eliminates the effects of electrode poisoning.

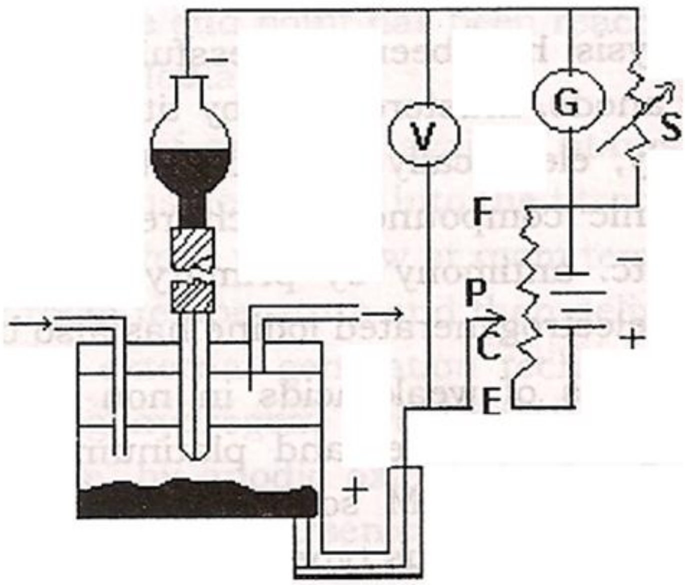
Check Your Progress – 5

1. What is uses of polarography?
2. Draw down the setup for polarography?
3. Draw down the graph of polarogram?
4. What is the most optimum for interval between drops in DME?
5. Write the any two advantage of DME?

5.8 ANSWER TO CHECK YOUR PROGRESS QUESTION

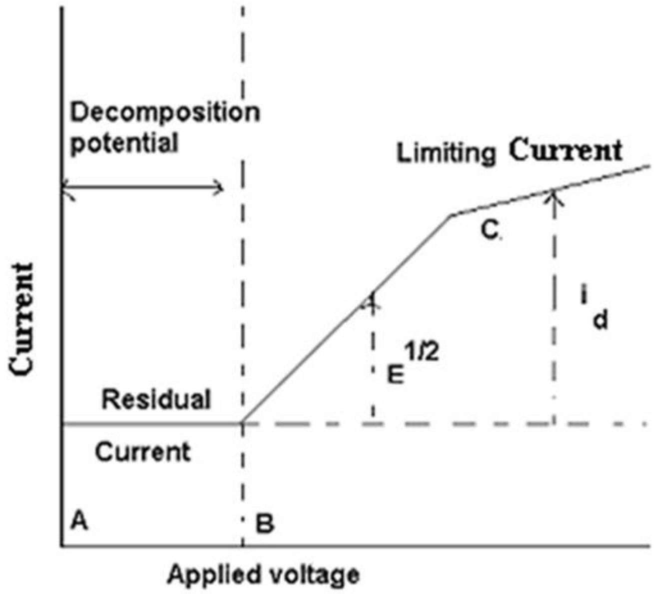
1. Polarography an electrochemical method for qualitative analysis, quantitative analysis, and the study of kinetics in chemical processes.
2. Setup for polarography

NOTES



NOTES

3. Graph of polarogram



4. The optimum interval between drops for most analyses is between 2 and 5 s.
5. (a) Mercury has a high hydrogen overvoltage. (a) It provides a smooth, fresh surface for the reaction.

5.9 SUMMARY

In polarography, the electric potential (i.e. voltage) of a growing mercury drop in an electrolyte containing an electroactive species is varied as a function of time and the resulting current due to the electrochemical reaction is measured. The technical elegance of this method is derived from several special properties of mercury as an electrode material. The fact that mercury is a liquid metal provides several advantages such as excellent renewability and reproducibility of the surface. This metal has also a wide potential range of operation in aqueous solution due to its large hydrogen overpotential. This unit 5 briefly deals about introduction, types and applications of polarography. Also, deals a specified polarography called dropping mercury electrode and its advantages

5.10 KEY WORDS

- Polarography
- Polarogram
- Current
- Types of Polarography
- Dropping mercury electrode

5.11 SELF-ASSESSMENT QUESTION AND EXERCISES

1. What is polarogram?
2. What is diffusion current?
3. What are the factors affecting the limiting current?
4. Write the types of polarography?
5. Draw the setup of dropping mercury electrode?
6. Write the main advantages of DME?

5.12 FURTHER READINGS

1. A. M. Bond, Modern Polarographic Methods in Analytical Chemistry, Marcel Dekker, New York (1980).
2. L. I. Antropov, Theoretical Electrochemisty, Mir Publishers, Moscow (1972).
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Unit 6: CYCLIC VOLTAMMETRY

Structure

6.1 INTRODUCTION

Cyclic voltammetry is a method for investigating the electrochemical behavior of a system. It was first reported in 1938 and described theoretically by Randies. Cyclic voltammetry is the most widely used technique for acquiring qualitative. Information about electrochemical reactions. The power of cyclic voltammetry results from its ability to rapidly provide considerable information on the thermodynamics of redox processes, on the kinetics of heterogeneous electron-transfer reactions, and on coupled chemical reactions or adsorption processes. Cyclic voltammetry is often the first experimental approach performed in an electroanalytical study, since it offers rapid location of redox potentials of the electroactive species and convenient evaluation of the effect of media upon the redox process Instructors of instrumental analysis who want to teach modern electrochemistry are faced with a similar dilemma due to the lack of suitable background material for the students to read. This unit 6 is intended to help meet those needs.

6.2 OBJECTIVES

After going through this unit, you will be able to

- To know the basic of AC polarography
- To know the what is cyclic voltammetry
- To determine the reversibility of electron transfer
- To understand the applications of cyclic voltammetry.

6.3 AC POLAROGRAPHY

Alternating current (AC) polarography is carried out by means of a conventional polarographic circuit together with a source of alternating voltage and a device for measuring the alternating current (Figure 6.3.1). C represents the cell system comprising dropping-mercury electrode, reference electrode, salt bridges, etc.; P, a source of e.m.f, and suitable voltage-divider, supplies the polarizing potential; the direct current is measured by means of the galvanometer G; S represents a source of alternating voltage and M a device for measuring the alternating current.

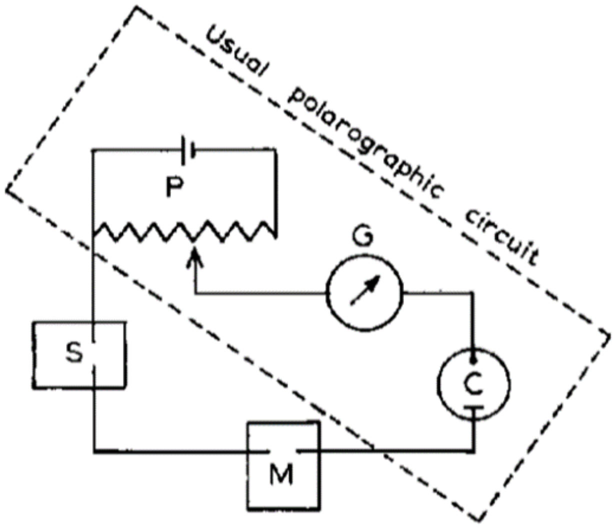
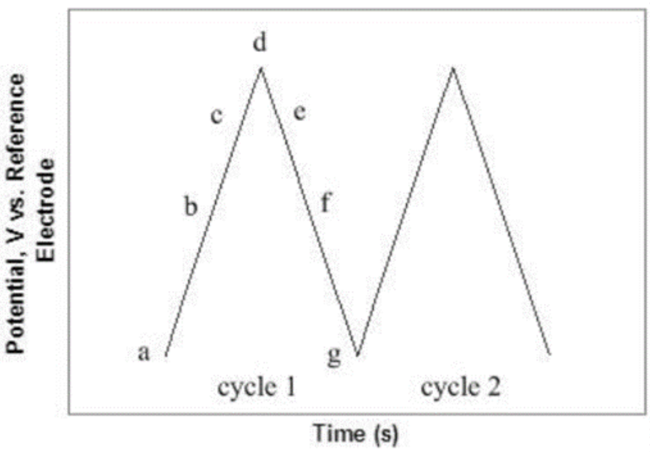


Figure 6.3.1; Schematic circuit diagram for AC polarography

6.4 CYCLIC VOLTAMMETRY

Cyclic Voltammetry (CV) is an electrochemical technique which measures the current that develops in an electrochemical cell under conditions where voltage is in excess of that predicted by the Nernst equation. CV is performed by cycling the potential of a working electrode, and measuring the resulting current.

The potential of the working electrode is measured against a reference electrode which maintains a constant potential, and the resulting applied potential produces an excitation signal such as that of Figure 6.4.1. In the forward scan of Figure 6.4.1, the potential first scans negatively, starting from a greater potential (a) and ending at a lower potential (d). The potential extrema (d) is call the switching potential, and is the point where the voltage is sufficient enough to have caused an oxidation or reduction of an analyte. The reverse scan occurs from (d) to (g), and is where the potential scans positively. Figure 6.4.1 shows a typical reduction occurring from (a) to (d) and an oxidation occurring from (d) to (g). It is important to note that some analytes undergo oxidation first, in which case the potential would first scan positively. This cycle can be repeated, and the scan rate



can be varied. The slope of the excitation signal gives the scan rate used.

Figure 6.4.1; CV Excitation Signal

6.5 INSTRUMENTATION

Cyclic voltammetry requires a waveform generator to produce the excitation signal, a potentiostat to apply this signal to an electrochemical cell, a current-to-voltage converter to measure the resulting current and an XY recorder or oscilloscope to display the voltammogram. The first three items are normally incorporated into a single electronic device although modular instruments are also used. The potentiostat insures that the working electrode potential will not be influenced by the reaction(s) which takes place. Data are typically obtained via XY recorder at slow scans, i.e. less than 500mV and storage oscilloscope at faster rates. Scan rates up to 20 mV/s have been used. However, rates faster than 100 mV/s are rarely practical because of iR drop and charging current.

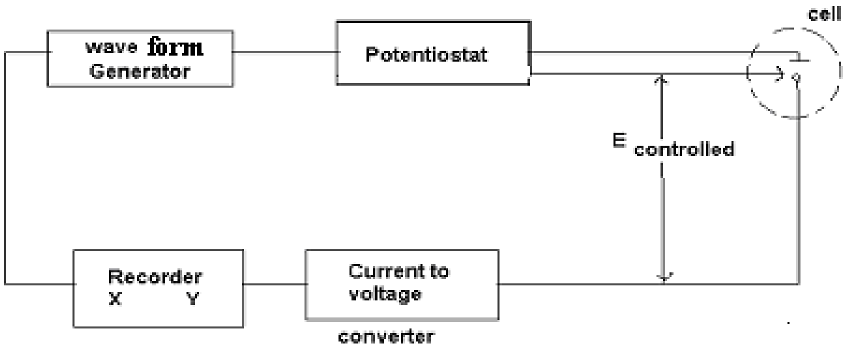


Figure 6.5.1: Instrumental set up for cyclic voltammetry

Modern potentiostat utilizes a three – electrode configuration as shown in Figure 6.5.1. The potentiostat applies the desired potential between a working electrode and a reference electrode. The working electrode is the electrode at which the electrolysis of interest takes place. The auxiliary electrode provides the current required sustaining the electrolysis at the working electrode. This arrangement prevents large current from passing through the reference electrode that could change its potential.

A typical electrochemical cell is illustrated in Figure 6.5.2. Such a cell usually consists of a glass container with a cap having holes for introducing electrodes and nitrogen. Provision is made for oxygen removal from solution by bubbling with nitrogen gas. The cell is then maintained oxygen free by passing nitrogen over the solution. The reference electrode is typically a SCE or Ag/AgCl electrode which often is isolated from the solution by a salt bridge to prevent contamination by leakage from the reference electrode. The auxiliary electrode is usually a platinum wire that is placed directly into the solution. Since the limiting (or peak) current in any type of voltammetry is temperature-dependent, the cell should be thermostated. Cell is commercially available which require as little as 1-2 ml of solution. Thin-layer cells enable voltammogram to be recorded on down to 60µL. a large verity of working electrodes has been used with voltammetry. The voltammetric techniques termed polarography utilize the dropping mercury electrode (DME). This electrode consist of mercury drop electrode (HMDE) is commonly used for CV. Here a drop of mercury can

be deposited on a substrate such as graphite to form a mercury is its good negative potential range.

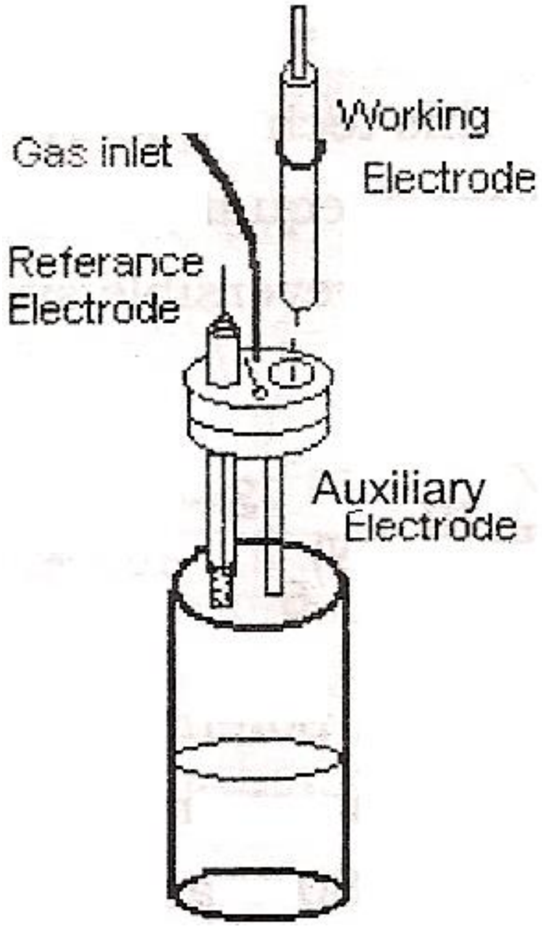


Figure 6.5.2: Cell set up for cyclic voltammetry

Solid electrodes such a platinum, gold, glassy carbon, wax impregnated graphite, and carbon paste are also commonly used in CV. Such electrode has a better positive potential range than mercury.

Scanning the potential in both directions provides us with the opportunity to explore the electrochemical behavior of species generated at the electrode. This is a distinct advantage of cyclic voltammetry over other voltammetric techniques.

6.6 TEST OF REVERSIBILITY OF ELECTRON TRANSFER REACTIONS

A typical cyclic voltammogram is shown in Figure 6.6.1 for a platinum working electrode in a solution containing 6.0M $K_3Fe(CN_6)$ as the electroactive species in 1.0 M KNO_3 in water as the supporting electrolyte.

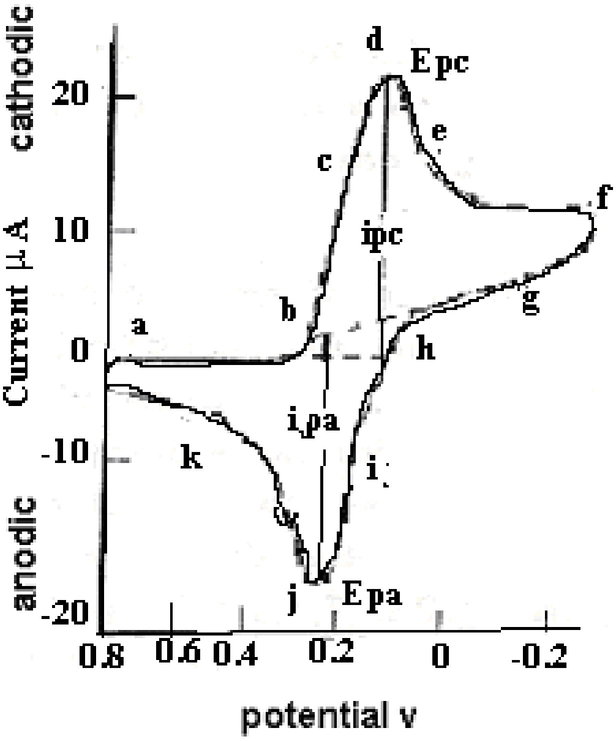


Figure 6.6.1: Diagram of typical cyclic voltammogram

(E_{pa}= anodic potential, E_{pc} = cathodic potential, i_{pa} = anotic peak current and i_{pc} = cathodic peak current)

The initial potential (E_i) of 0.80V applied at (a) is chosen to avoid any electrolysis of Fe $(CN_6)^{3-}$ when the electrode is switched on. The potential is then scanned negatively, forward scan, as indicated by the arrow. When the potential is sufficiently negative to reduce FeIII $(CN_6)^{3-}$, cathodic current is indicated at (b) due to the electrode process

The electrode is now a sufficiently strong reductant to reduce FeIII $(CN_6)^{3-}$. The cathodic current increases rapidly until the concentration of FeIII $(CN_6)^{3-}$ at the electrode surface is substantially diminished, causing the current to peak (d). The current then decays (d-g) as the solution surrounding the electrode is depleted of FeIII$(CN_6)^{3-}$, due to its electrolytic conversion to FeIII$(CN_6)^{4-}$. The scan direction is switched to positive at -0.15V (f) for the reverse scan. The potential is still sufficiently negative to reduce FeIII $(CN_6)^{3-}$, so cathodic current continues even though the potential is now scanning in the positive direction. When the electrode becomes a sufficiently strong oxidant, FeIII $(CN_6)^{4-}$, which has been accumulating adjacent to the electrode, can now be oxidized by the electrode process.

This causes anodic current (i-k) the anodic current rapidly increase until the surface concentration of FeIII$(CN_6)^{4-}$, is diminished. Causing the current to peak (j) the current then decay (j-k) as the solution surrounding the electrode is depleted of FeIII$(CN_6)^{4-}$. The first cycle is completed when the potential reaches +0.8V. Now the cyclic voltammogram graph is obtained. It is apparent that any potential positive of ~+0.4V would be suitable as an initial potential in that reduction of FeIII$(CN_6)^{3-}$ would not occur when the potential. In the forward scan FeIII$(CN_6)^{4-}$ is

electrochemically generated from $FeIII(CN_6)^{4-}$, as indicated by the cathodic current. In the reverse scan

this $FeIII(CN_6)^{4-}$, is oxidized back to $FeIII(CN_6)^{4-}$, as indicated by the anodic current. Thus, CV is capable of rapidly generating a new oxidation state during the forward scan and the probing its fate on the reverse scan. This is the chief strength of this technique. A more detailed understanding can be gained by considering the Nernst equation and the changes in concentration described by the Nernst equation for a reversible system. By analysing the variation of peak position as a function of scan rate it is possible to gain an estimate for the electron transfer rate constants.

6.7 APPLICATIONS

- Cyclic Voltammetry can be used to study qualitative information about electrochemical processes under various conditions, such as the presence of intermediates in oxidation-reduction reactions, the reversibility of a reaction.
- CV can also be used to determine the electron stoichiometry of a system, the diffusion coefficient of an analyte, and the formal reduction potential, which can be used as an identification tool.
- In addition, because concentration is proportional to current in a reversible, Nernstian system, concentration of an unknown solution can be determined by generating a calibration curve of current vs. concentration.

Areas of chemistry	Typical applications
Analytical chemistry	Preparation and synthesis
	Analysis
	Mechanism
Inorganic chemistry	Metal interactions and reactions
	Structure characterizations
	Analysis
Organic chemistry	Synthesis
	Analysis
	Characterization
	Mechanism
Physical chemistry	Thermodynamic studies
	Kinetic studies
	Theoretical equations
	Surface and adsorption
	Analysis
	Mechanism
Biochemistry (and biology)	Metabolic processes
	Continuous monitoring
	In vivo and in vitro analysis
	Kinetic of enzymes
	Biosensors

Table 6.7.1 Summarized applications of CV in all aspects of chemistry

Cyclic
Voltammetry

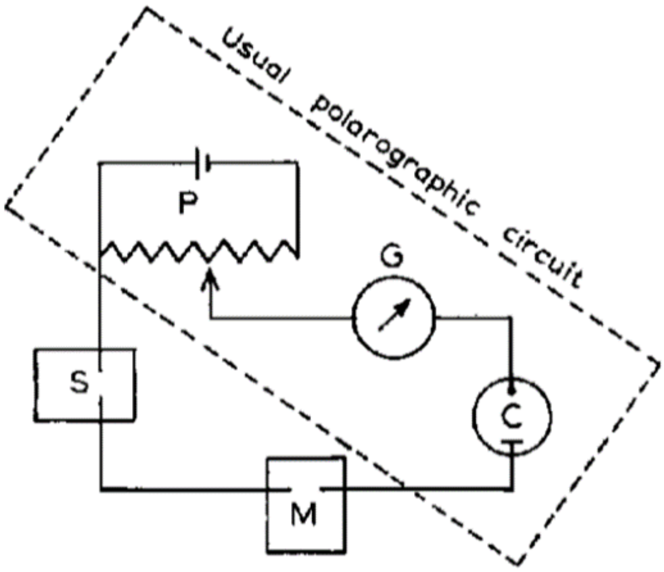
NOTES

Check Your Progress – 6

1. Define alternating current polarography?
2. Draw the schematic circuit diagram for AC polarography.
3. What are working electrodes commonly used in CV measurement?
4. How did the concentration of an unknown solution determine by CV?
5. Mention the any three typical applications of CV in analytical chemistry?

6.8 ANSWER TO CHECK YOUR PROGRESS QUESTION

1. Alternating current (AC) polarography is carried out by means of a conventional polarographic circuit together with a source of alternating voltage and a device for measuring the alternating current
2. Schematic circuit diagram for AC polarography



3. Solid electrodes such a platinum, gold, glassy carbon, wax impregnated graphite, and carbon paste are also commonly used in CV.
4. Because concentration is proportional to current in a reversible, Nernstian system, concentration of an unknown solution can be determined by generating a calibration CV curve of current vs. concentration.
5. Preparation and synthesis, analysis and mechanism.

6.9 SUMMARY

In summary, cyclic voltammetry is a convenient tool for obtaining qualitative information about electron transfer processes. It is also a rapid method for obtaining good estimates of formal reduction potentials, formation constants and, sometimes, the number of electrons transferred per reactant molecule and rate constants, if the user is aware of its limitations.

6.10 KEY WORDS

- AC-polarography
- Cyclic voltammetry
- Cyclic voltammogram
- Electron transfer reaction
- Peak potential

6.11 SELF-ASSESSMENT QUESTION AND EXERCISES

1. What is cyclic voltammetry?
2. Draw the cell set-up for cyclic voltammetry.
3. Write the advantage of cyclic voltammetry over other voltammetric techniques?
4. Draw the diagram of typical cyclic voltammogram for 6.0 M $K_3Fe(CN_6)$.
5. How potential of the working electrode measured by CV?
6. Mention the any three typical applications of CV in inorganic chemistry?

6.12 FURTHER READINGS

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3. Carriedo, Gabino A. "The use of cyclic voltammetry in the study of the chemistry of metal-carbonyls: An introductory experiment." *J. Chem. Educ.* 1988, *65*, 1020.

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Unit 7: CHRONO TECHNIQUES

Structure

7.1 INTRODUCTION

Chronoamperometry, chronocoulometry, and chronopotentiometry belong to the family of step techniques. In chronoamperometry the current, while in chronocoulometry the charge is measured as a function of time after application of a potential step perturbation. Chronopotentiometry is a method in which the controlled variable is the current. In this method the current is suddenly stepped from a zero value to some preset value, and the change in the potential of the electrode recorded as a function of time. Chronocoulometry is the measurement of charge per unit time. In this technique the experiment starts at a potential where no reaction is occurring and stepped instantaneously to a potential where either the oxidation or reduction occurs.

7.2 OBJECTIVES

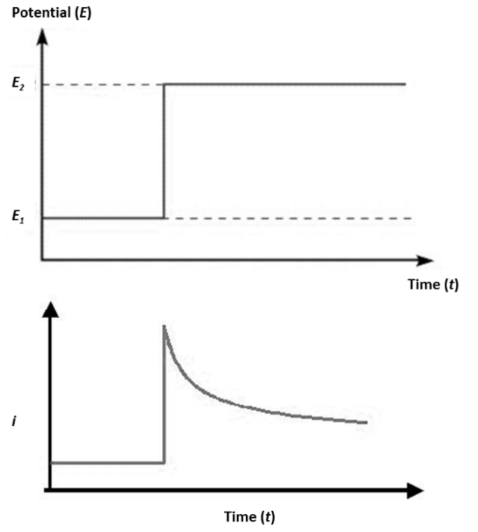
After going through this unit, you will be able to

- ❖ To know the basic concept of controlled techniques
- ❖ To know the basic of chronopontential
- ❖ To understating the controlled potential coulometry
- ❖ To define the electrogravimetry.

7.3 CHRONO TECHNIQUES

Chorono techniques also called as controlled techniques in which the potential (*E*) or current (*i*) of an electrode was controlled, while the current or potential was determined as a function of time. Chorono techniques was mainly classified in to two methods such as (a) chronoamperometry (controlled potential technique) and (b) chronopotentiometry (controlled current technique).

(a) In the chronoamperometry, the current was determined as a function of time while the potential of the electrode was controlled



(Figure 7.3.1).

Figure 7.3.1: Simplest experimental diagram of chronoamperometry.

(b) In the chronopotentiometry, the potential was determined as a function of time while the current of the electrode was controlled (Figure 7.3.2).

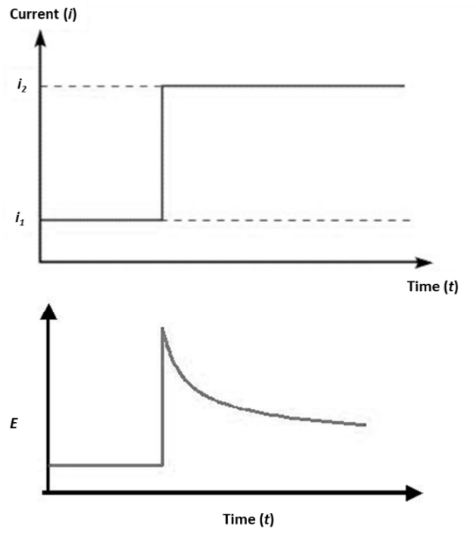


Figure 7.3.2: Simplest experimental diagram of chronopotentiometry.

7.4 CHRONOPOTENTIOMERTY

The experiment is carried out by applying the controlled current between the working and auxiliary electrodes with a current source (called a galvanostatic) and recording the potential between the working and reference electrodes (e.g., with a recorder, oscilloscope, or other data acquisition device) (Figure 7.4.1). These techniques are generally called chronopotentiometric techniques, because *E* is determined as a function of time, or galvanostatic techniques, because a small constant current is applied to the working electrode. A simple circuit of chronopotentiometric techniques shown in Figure 7.4.2.

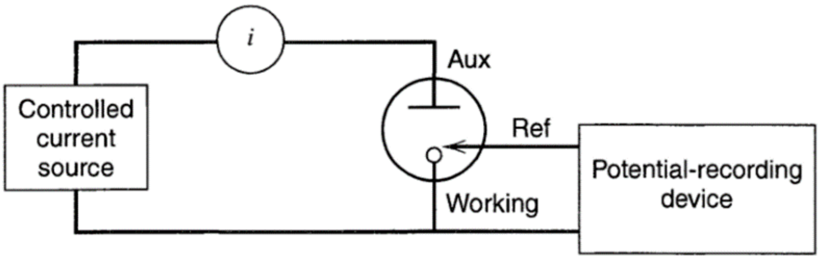


Figure 7.4.1: Simplified block diagram of apparatus for chronopotentiometric measurements.

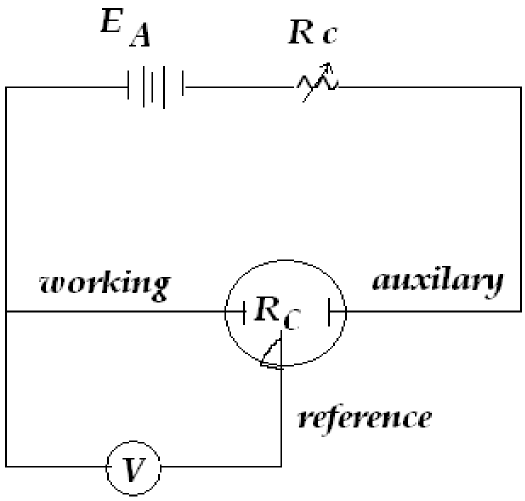


Figure 7.5.2: Circuit for Chrono technique

In this circuit E_A represents the applied voltage and R_L us current-limiting resistor in series with the cell. If R_C is the resistance of the cell and the back e.m.f of the cell is neglected then the current is represented by E_A/R_L+R_C.

7.5 ADVANTAGE OF CHRONOPOTENTIOMETRY

- Chronopotentiometry methods can be of particular value when the process being studied is the background process, such as solvated electron formation in liquid ammonia or reduction of quaternary ammonium ion in an aprotic solvent.
- A simple method for determination of the thickness of metal films is by anodic stripping at constant current.

➢ Working with background processes in a controlled-potential mode is often difficult.

7.6 CONTROLLED POTENTIAL COULOMETRY

The easiest way to ensure 100% current efficiency is to hold the working electrode at a constant potential, chosen so that the analyte reacts completely without simultaneously oxidizing or reducing an interfering species. As electrolysis progresses the analyte's concentration decreases, as does the current. The resulting current-versus-time profile for controlled-potential coulometry is shown in Figure 7.6.1. Integrating the area under the curve from t = 0 to t = t_e gives the total charge. In this section we consider the experimental parameters and instrumentation needed to develop a controlled-potential coulometric method of analysis.

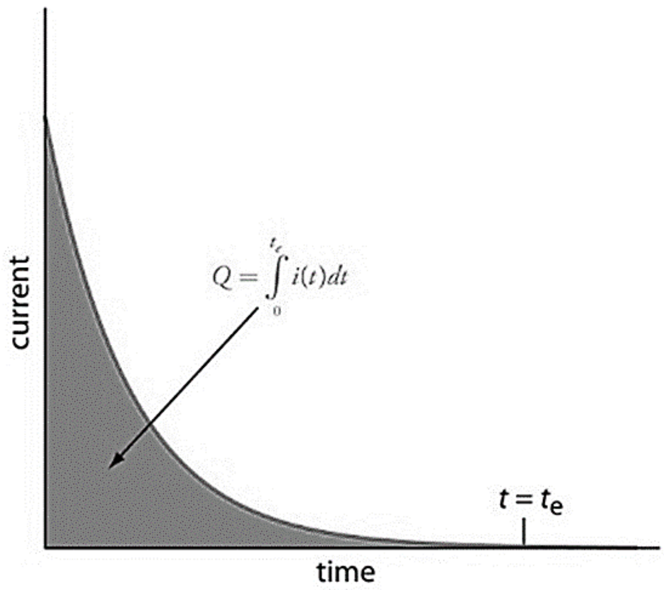


Figure 7.6.1: Current versus time for a controlled-potential coulometric analysis

7.6.1 Instrumentation

A three-electrode potentiostat is used to set the potential in controlled-potential coulometry. The working electrodes is usually one of two types: a cylindrical Pt electrode manufactured from platinum-gauze, or a Hg pool electrode. The large overpotential for the reduction of H_3O^+ at Hg makes it the electrode of choice for an analyte requiring a negative potential. For example, a potential more negative than –1 V versus the SHE is feasible at a Hg electrode but not at a Pt electrode even in a very acidic solution. Because mercury is easily oxidized, it is less useful if we need to maintain a potential that is positive with respect to the SHE. Platinum is the working electrode of choice when we need to apply a positive potential.

The auxiliary electrode, which is often a Pt wire, is separated by a salt bridge from the analytical solution. This is necessary to prevent the electrolysis products generated at the auxiliary electrode from reacting with the analyte and interfering in the analysis. A saturated calomel or Ag/AgCl electrode serves as the reference electrode.

The other essential instrumental need for controlled-potential coulometry is a means for determining the total charge. One method is to monitor the current as a function of time and determine the area under the curve, as shown in Figure 7.6.1. Modern instruments use electronic integration to monitor charge as a function of time. The total charge at the end of the electrolysis is read directly from a digital readout.

7.6.2 Electrogravimetry

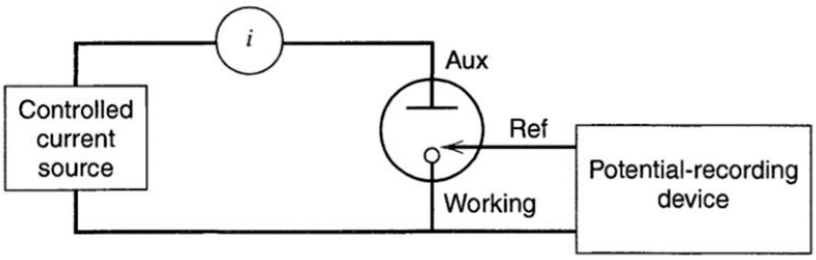
If the product of controlled-potential coulometry forms a deposit on the working electrode, then we can use the change in the electrode's mass as the analytical signal. For example, in we apply a potential that reduces Cu^{2+} to Cu at a Pt working electrode, the difference in the electrode's mass before and after electrolysis is a direct measurement of the amount of copper in the sample. We call an analytical technique that uses mass as a signal a gravimetric technique; thus, we call this electrogravimetry.

Check Your Progress – 7

1. What is chronotechniques?
2. Which was determined by chronopotentiometry? How?
3. Which was determined by chronoamperometry? How?
4. Draw the simplified block diagram of apparatus for chronopontentiometric measurements?
5. Write down the types of working electrodes possible to use in controlled-potential coulometry?

7.7 ANSWER TO CHECK YOUR PROGRESS QUESTION

1. Chorono techniques also called as controlled techniques in which the potential (*E*) or current (*i*) of an electrode was controlled, while the current or potential was determined as a function of time.
2. In the chronopotentiometry, the potential was determined as a function of time while the current of the electrode was controlled.
3. In the chronoamperometry, the current was determined as a function of time while the potential of the electrode was controlled.
4. Simplified block diagram of apparatus for chronopotentiometric measurements



5. The working electrodes is usually one of two types in controlled-potential coulometry: a cylindrical Pt electrode manufactured from platinum-gauze, or a Hg pool electrode.

7.8 SUMMARY

In summary, unit 7 briefly deals about controlled techniques, in particularly chronopotentiometry and chronocoulometry (controlled potential coulometry). Also it deals about the instrumentation and advantages of chronopotentiometry and basic instrumentation over chronocoulometry.

7.9 KEY WORDS

- Chronotechniques
- Controlled potential method
- Chronopotentiometry
- Controlled potential coulometry
- Electrogravimetry

7.10 SELF-ASSESSMENT QUESTION AND EXERCISES

1. Write the main classification of controlled techniques?
2. Define chronoamperometry?
3. What is chronopotentiometry?
4. Write any two advantages of chronopotentiometry?
5. Define Controlled potential coulometry?
6. Write down the concept of electrogravimetry?

7.11 FURTHER READINGS

1 Boris B. Damaskin, "The Principles of Current Methods for the Study of Electrochemical Reactions", Editor, Gleb Maamntov, McGraw-Hill Book Co., New York, (1968).

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BLOCK-III
CHROMATOGRAPHY

8. CHROMATOGRAPHIC METHODS

Structure

8.1 INTRODUCTION

The first purely pragmatic application of chromatography was that of the early dye chemists, who tested their dye mixtures by dipping strings or pieces of cloth or filter paper into a dye vat. The dye solution migrated up the inserted material by capillary action, and the dye components produced bands of different colour. In the 19th century, several German chemists carried out deliberate experiments to explore the phenomenon giving it the name "capillary analysis." The discovery of chromatography, however, is generally attributed to the Russian botanist Mikhail S. Tsvet, because in 1901 he recognized the physicochemical basis of the separation and applied it in a rational and organized way to the separation of plant pigments, particularly the carotenoids and the chlorophylls.

8.2 OBJECTIVES

After going through this unit, you will be able

- ❖ To know the classification of chromatography
- ❖ To know the elementary principle involved in paper chromatography
- ❖ To know gain knowledge on principle of thin layer chromatography (TLC).
- ❖ To understand more on experimental techniques and application of TLC.

8.3 DEFINITION

Chromatography is an analytical technique, which is employed to separate the components of a mixture into its individual components on the basis of their equilibrium distribution between two phases. In other words, it is a technique for separating the components, or solutes, of a mixture on the basis of the relative amounts of each solute distributed between a moving fluid stream, called the mobile phase, and a contiguous stationary phase. The mobile phase may be either a liquid or a gas, while the stationary phase is either a solid or a liquid.

Kinetic molecular motion continuously exchanges solute molecules between the two phases. For a particular solute, the distribution favours the moving fluid, the molecules will spend most of their time migrating with the stream and will be transported away from other species whose molecules are retained longer by the stationary phase. For a given species, the ratio of the times spent in the moving and stationary regions is equal to the ratio of its concentrations in these regions, known as the partition coefficient. A mixture of solutes is introduced into the system in a confined region or narrow zone (the origin), whereupon the different species are transported at different rates in the direction of fluid flow. The driving force for solute migration is the moving fluid, and the resistive force is the solute affinity for the stationary phase; the combination of these forces, as manipulated by the analyst, produces the separation.

8.4 CLASSIFICATION OF CHROMATOGRAPHY

In general, chromatographic methods are classified according to mobile phase (gas or liquid), stationary phases (solid or liquid film on support), and separation mechanism (adsorption, partition, ion exchange, permeation). Here the chromatographic methods are classified into six combinations of phase and processes giving rise to a large number of methods with individual names as shown in table 8.4.1.

Table 8.4.1: Classification of chromatographic methods.

Stationary phase	Mobile phase	Name
Solid	Liquid	Plane Chromatography Paper Chromatography Thin Layer Chromatography Adsorption Column Chromatography High Performance Liquid Chromatography
Solid (ion exchange resin)	Liquid	Ion Exchange Chromatography
Solid	Gas	Gas – Solid Chromatography
Solid matrix	Liquid	Gel Permeation Chromatography
Liquid	Gas	Gas-Liquid Chromatography
Liquid	Liquid	Liquid-Liquid Chromatography

8.5 BASIC AND ELEMENTARY PRINCIPLE AND PRACTICE OF PAPER CHROMATOGRAPHY

8.5.1 Principle

Paper chromatography, in analytical chemistry, technique for separating dissolved chemical substances by taking advantage of their different rates of migration across sheets of paper. It is an inexpensive but powerful analytical tool that requires very small quantities of material. It is a type of partition chromatography in which the substances are distributed between two liquids, one is the stationary phase which is held in the fibres of the paper, and other is, developing solvent, called moving phase. The extent of migration of a compound is represented by its R_f value

$$R_f = \frac{\text{Distance traversed by the component from the origin line}}{\text{Distance traversed by the solvent from the origin line}}$$

The $R_{f\ value}$ of a compound depends on the solvent, medium, temperature and nature of the mixture. In some cases, the solvent front runs off the end of filter paper. In such cases the movement of substance is expressed R_x

$$R_x = \frac{\text{Distance traversed by the component from the origin line}}{\text{Distance traversed by the standard substance from the origin line}}$$

Experimentally the paper chromatography is performed for two type of analysis namely qualitative analysis and quantitative analysis

8.5.2 Qualitative Analysis

Selection of the chromatographic mode

The mode of technique used for the separation (ascending, descending etc.) depends on the nature of substances to be separated.

Choice of filter paper

The choice of filter paper depends on the following the factors:

1. Whether it is used for quantitative or qualitative analysis
2. Whether it is used for analytical or preparative chromatography
3. The nature of the substances (hydrophilic, lipophilic, neutral or charged species.

Generally used Whatman filter papers for chromatography techniques have 99%α-cellulose. The capillarity of the paper is increased by the partial hydrolysis by soaking the filter paper for 24 hours in 7% hydrochloric acid and washing successively with water and ethanol are used in paper chromatographic technique.

Solvent system

The choice of solvent depends on R_f value which is various for different compounds present in the mixture. The factors governing the good solvent system are

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1. The R_f values of sample should lie between 0.05 and 0.85 in the system
2. The difference between the R_f values of any two components must be 0.05
3. The distribution ratios of the components in the solvent system should be independent of concentration
4. The solvents should not undergo chemical reaction with the components of the sample mixture, and
5. The composition of the solvent system should not alter with time.

If single solvent is not satisfactory, suitable polarity may be obtained by mixing two or three solvents in various proportions.

Spotting of the sample

For ascending technique (fig.3.19.1), Whatman filter paper of suitable size usually 25 cm X 7 cm is used. The origin line is drawn with the lead pencil. With the help of graduated micro pipette, the test solution is applied on the origin line. Each spot should be at least 2cm away from each other.

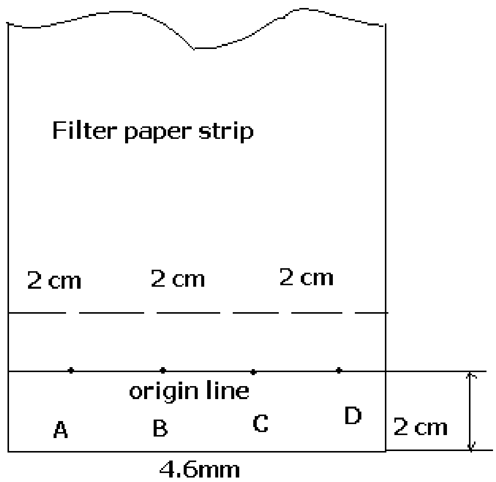


Figure 3.19.1: Diagram of Spotting and chromatographic paper

After the development, the wet chromatograms are dried using special drying cabinet.

Identification of the spots & calculate R_f value

In the chemical method, the colourless spots are converted in to the coloured one with the help of certain reagents, which are known as chromogenic reagents. In physical method UV lamp is used for the identification of the components. Finally the R_f values are calculated for the separated components.

8.5.3 Quantitative Analysis

For this analysis, the separation is carried out the same way as in the qualitative analysis. The estimation can be done after the extraction of the substance from the paper or by in situ on the paper.

1. Extraction of separated compounds from paper chromatography Cut out a part of the paper containing the spot and soak it in the minimum

quantity of the solvent. A semi-micro extractor is being used for the extraction. The separation of the spot is known as elution.

2. Determination- The microanalysis of the resultant eluate can be carried out by one or more of the following techniques:

I. Gravimetric estimation
II. Colorimetry
III. Coulometry
IV. Flame photometry
V. UV-spectrophotometry
VI. Polarography
VII. Radioactivity

8.5.4 Applications of paper chromatography

Paper chromatography has been used for the separation of many organic and biochemical products. Example – It has been used in the determination of indoles in urine and in the study of barbiturates, antibiotics, carbamoyl phosphates, and amino acids. It has also been used in the study of inorganic metal salts and complex ions. Paper chromatography has become standard practice for the separation of complex mixtures of amino acids, peptides, carbohydrates, steroids, purines, and a long list of simple organic compounds. Inorganic ions can also readily be separated on paper.

8.6 THIN-LAYER CHROMATOGRAPHY

The technique of was first introduced by Izmailov and Shraiber in 1938. Attempts were made by Kirchnen in 1950 that carried out adsorption Chromatography on impregnated filter paper and later on glass-fine paper coated with silicic acid or alumina. Stahl was the one introduced TLC as a procedure for analytical adsorption Chromatography. TLC is similar to paper chromatography, instead of paper, a thin layer (0.25mm) of inert material such as Al_2O_3, MgO or SiO_2 is used as the substrate. Other names for TLC are drop, strip, spread layer, surface chromatography and open column chromatography.

8.6.1 Principle

Thin-layer chromatography (TLC) is a technique for separating dissolved chemical substances by virtue of their differential migration over glass plates or plastic sheets coated with a thin layer of a finely ground adsorbent, such as silica gel or alumina, that is mixed with a binder such as starch or plaster of Paris.

8.6.2 Experimental Techniques

Adsorbents

The governing factors for the selection of the adsorbent depend on the solubility, nature (whether it is basic, acidic or amphoteric) and the inertness (inert towards the adsorbent or solvent) of the substance to be separated. The following table 8.6.2.1 contains the details of generally used adsorbents.

Table 8.6.2.1: List of absorbents used for TLC

Name of Adsorbent	Acidic / Basic	Activity	Separatory Mechanism	Compounds to be separated
Silica gel	Acidic	Active	Adsorption Partition	Acidic and neutral substances
Alumina	Basic	Active	Adsorption Partition	Basic and neutral
Kieselguhr	Neutral	Inactive	Partition	Strongly hydrophilic Substances
Cellulose powder	Neutral	None	Partition	Water soluble compounds

A particle size of 1-25 microns is usually used for the separations due to the fact that fine grained particle is one of the reasons for the enhanced resolution in TLC.

Adsorbents do not adhere very well to the glass plates. Various types of binders are added to overcome this problem. Calcium sulphate is commonly used as a binder. Other binding materials are starch, hydrated silicon oxide, etc., Chemically inert fluorescent indicator such as zinc silicate is added in the adsorbents of the precoated plates which helps in the identification of non-fluorescent but UV absorbing substances.

Preparation of TLC plates

The TLC plates consist of thin layers of adsorbent on a base made of glass, aluminium foil or plastic. The advantages of glass plates are (i) they can be easily cleaned and (ii) reused. The aqueous slurry of adsorbent is applied to the plate and dried. The thickness of the adsorbent is usually 100-300 micron. The various methods of preparing thin layers are as follows.

Pouring

A measured quantity of the slurry is poured on a given size of plate and the slurry is spread uniformly over the surface.

Dipping

In this method TLC plates are prepared by dipping them at a time, back to back in chloroform or chloroform methanol slurries of the adsorbent.

Spreading

The slurry is placed in an applicator. The apparatus consists of two pairs. In the aligning tray the plates are set in a line and spreader takes up the spreading mixture and applies it uniformly on a thin layers. This method is used to get thin and uniform layer. The method of operation is shown Figure 8.6.2.1

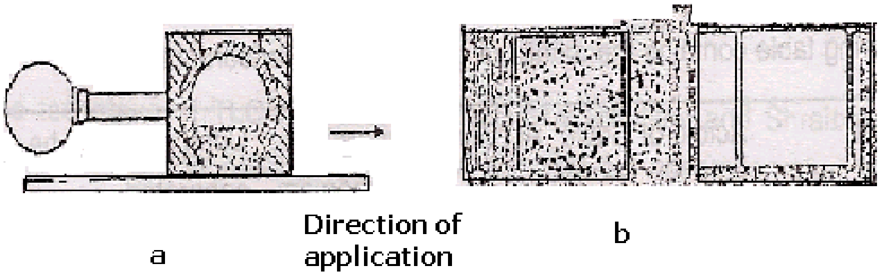


Figure 8.6.2.1: (a).The operation of TLC spreader and (b). Aligning tray with glass slide partially coated

Precoated plates

Common adsorbents precoated on glass or plastic sheets are available in the market. The thickness of the plates varies from 0.1 to 0.2 mm.

Activation of Adsorbent

The adsorbent is activated by the removal of the liquid completely which is associated with the thin layer by drying the plate for 30 minutes in air and then in over at 110°C for another 30 minutes. The heating silica and alumina plates at 150°C for 4 hours results for very active layers.

Purification of silica gel 'G' layers

Iron present in the silica gel a causes distortion of chromatographs. It has been removed by the preliminary development of the coated and air-dried plates with methanol-Con. HCl (9:1v/v). The iron moved with the solvent front to the upper edges of the plate. The plates are again dried and activated at 110°C.

Sample application

Micro syringe is used for the sample application for qualitative work. In some cases, capillary tubes may also be used.

Development chamber

The development chamber used for paper chromatography can also be used for TLC. The common technique employed in TLC is ascending. The plate is placed in the tank at an angle of 45°C. The chamber is filled with the solvent at the bottom up to nearly 1mm. Three sides of the tank are covered with solvent impregnated paper and the top should be covered tightly with the lid. The chamber should be saturated with solvent vapours to avoid unequal solvent evaporation losses from the developing plate (Figure 8.6.2.2).

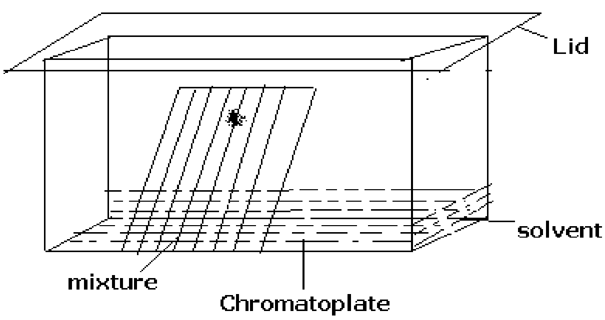


Figure 8.6.2.2: Apparatus set up for TLC

Solvent

The choice of the solvent depends on the nature of the substance to be separated. Mixtures of two or more solvents of different polarity often give better separation than chemically homogeneous solvents. The solvents used should be pure. A series of solvents beginning with the solvent of highest non polar properties is given below. Petroleum ether – cyclohexane - toluene-chloroform - ether- ethyl acetate - n- butanol -phenol - acetone-ethanol-acetic acid - methanol-formamide-water.

Development of TLC plate

A drop of the sample solution is placed near one edge of the plate and its position is marked with the pencil. Now the sample solvent is evaporated and the TLC plate is placed in the development tank which is saturated with the solvent vapour. Solvent is allowed to rise to the height of about two-third the length of the plate, and then the plate is removed from the chamber and dried.

Identification of components

Colored spots can be identified visually. The colorless spots are identified by UV light or by the color development with the chromogenic reagents. The corrosive agents like sulphuric acid or chromic acid can also be used in TLC for the identification of the separated components which is not possible in the paper chromatographic technique.

Evaluation of Chromatogram

After identifying the separated solutes their evaluation may be either qualitative or quantitative. The qualitative evaluation in TLC is same as with the paper chromatogram. The quantitative evaluation of the separated components is carried out either by direct or indirect methods. Quantitative estimation is carried out directly on the layer by visual assessment, measurement of the spot area or by densitometry, etc.

8.6.3 Advantages

1. It requires simple equipment.
2. Development time is short (about 1 hour) compared to paper and column Chromatography which requires several hours or days.
3. There is a wide choice of stationary phase. It can be employed for adsorption partition or ion exchange Chromatography.
4. Early recovery of separated components. It is easy to remove the powdery coating of plates. The spot or zone may be removed quantitatively and required compound dissolved in a suitable solvent is determined by calorimetric or spectrophotometric analysis.
5. Effective resolution can be achieved by TLC than by paper chromatography
6. Under UV light, detection of fluorescence compounds is easier than on paper Chromatography.

7. Extremely sharp spots are obtained in TLC. The increase in sensitivity is of the order of 10 to 100 folds as compared to that of paper chromatography.

8. Corrosive reagents like sulphuric or chromic acid can be used. This is not possible in paper Chromatography

8.6.4 Application of TLC

Thin layer chromatography is used for the quantitative determination of high molecular weight compounds, particularly in medical and biological research. The various applications of TLC are as follows:

1. TLC has been used as a tool for checking the separation procedures and purification processes.
2. TLC has been used to assess the completion of the reaction and sometimes, the behavior of the spots with the standard reagents, will give information for the identification of the product.
3. TLC has been used for characterizing and isolating the organic compounds such as acids, alcohols, glycols, amino acids, amines, proteins, antibiotics, etc.,
4. TLC has been used for separating inorganic ions i.e. Cationic, anionic, covalent species and also some organic derivatives of the metals.
5. Adsorption TLC on absorbents has been employed to the fractionation of mixture of non-polar materials into classes of compounds that migrate at different rates by virtue of their respective polarities.
6. Ion exchange TLC technique the rate of migration of a compound is found by the total charge of the ionized groups per molecule.
7. Partition TLC technique cellulose powder and air-dried adsorbents are used as coating materials.
8. Reversed phase partition TLC technique is used for the separation of hydrocarbons and other non-polar petroleum products, fats etc.,

Check Your Progress – 4

1. What is chromatography?.
2. Write the applications of paper chromatography?
3. What does the choice of filter paper depends on?
4. Explain the principle of TLC?

8.7 ANSWER TO CHECK YOUR PROGRESS QUESTION

1. Chromatography is an analytical technique, used to separate the components of a mixture into its individual components based on their equilibrium distribution between two phases i.e. between the mobile and stationary phase.

2. It is used for the separation of many organic and biochemical products, the study of inorganic metal salts and complex ions

3. The choice of filter paper depends on the following the factors:
   - Whether it is used for quantitative or qualitative analysis
   - Whether it is used for analytical or preparative chromatography
   - The nature of the substances (hydrophilic, lipophilic, neutral or charged species).
4. Thin layer chromatography depends on the separation of the relative affinity of compounds towards stationary and the mobile phase compounds in the mobile phase travel over the surface of the stationary phase. During this movement, the compounds with higher affinity to stationary phase travel slowly while the others travel faster. Thus, the separation of components in the mixture is achieved.

8.8 SUMMARY

Chromatography is the collective term for the separation of mixtures. The various constituents of mixture travel at different speed causing the mixture to separates. It usually consists of mobile phase and stationary phase. It is used in the quantitative and qualitative analysis of complex mixture and in the determination of molecular weight of proteins etc. paper chromatography involves placing a small dot onto a strip of chromatography paper which is also referred to as a two-dimensional chromatography. TLC is a similar technique instead of using paper as stationary phase, it involves a thin layer of silica gel, alumina or Cellulose.

8.9 KEY WORDS

- Sample spotting
- Adsorbent
- Mobile phase
- Stationary phase

8.10 SELF-ASSESSMENT QUESTION AND EXERCISES

1. Discuss about the classification of chromatography?
2. What is mean by spotting of the sample?
3. What does the selection of chromatographic mode on the paper chromatography depends on?
4. Explain the experimental technique in TLC?
5. Write the applications of TLC?

8.11 FURTHER READINGS

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2. A.I Vogel, Text Book of Quantitative organic Analysis, ELBS III Edn, 1987.
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9. GAS CHROMATOGRAPHY

Structure

9.1 INTRODUCTION

Gas chromatography (GC) is a common type of chromatography used in analytical chemistry for separating and analysing compounds that can be vaporized without decomposition. Typical uses of GC include testing the purity of a particular substance, or separating the different components of a mixture (the relative amounts of such components can also be determined). In some situations, GC may help in identifying a compound. In preparative chromatography, GC can be used to prepare pure compounds from a mixture. German physical chemist Erika Cremer in 1947 together with Austrian graduate student Fritz Prior developed the theoretical foundations of GC and built the first liquid-gas chromatograph, but her work was deemed irrelevant and was ignored for a long time. The popularity of gas chromatography quickly rose after the development of the flame ionization detector.

9.2 OBJECTIVES

After going through this unit, you will be able

- ❖ To gain knowledge on Gas chromatographic techniques
- ❖ To know the principle involved in GC
- ❖ To develop knowledge on the GC instrumentation and know the advantages of GC.
- ❖ To know the applications of GC techniques

9.3 GAS CHROMATOGRAPHIC TECHNIQUES

It is a powerful tool for the separation of complex mixtures, because of the speed, extreme sensitivity and high resolving power of the technique. GC can be further divided into Gas-liquid Chromatography (GLC) and Gas-Solid Chromatography (GSC). In GLC the mobile phase is

a gas and the

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stationary phase is a thin layer of non-volatile liquid bound to a solid support. It is based on the principle that there is a partition between the gas and an immobile liquid phase. GSC consists of a solid adsorbent as a stationary phase and gas as a mobile phase. The adsorption process takes place here.

9.4 PRINCIPLE

When a gas or vapor comes in contact with an adsorbent, certain amount of it gets adsorbed on the solid surface. This adsorption takes place according to the Freundlich Law i.e., $x/m=Kc^{1/n}$ or Langmuir i.e. $x/m= K_1c + K_2c$. where x is the mass of the gas or vapor gets adsorbed in mass m of the adsorbent and c is the vapor concentration in the gas phase and Kc, K_1 and K_2 are constants. Similarly, if the vapor or gas comes in contact with liquid, a fixed amount of it is dissolved in the liquid. These phenomena take place according to Henry's law of partition, i.e. $x/m=Kc$. Both the phenomena are selective and there are different K values for different vapor-absorbent pairs.

In GLC the stationary liquid phase is coated on to a solid matrix or the wall of a capillary tube. The stationary phase has low vapor pressure at the column temperature, so it can be non-volatile. The sample mixture in gaseous form runs through the column with a carrier gas. Separation can be achieved by the difference in the distribution ratios of the components of the sample between the mobile and stationary phases causing them to move through the column at different rates and with different retention time. After elution, the sample components are detected at the exit by a suitable detector. If required, the components can be collected for preparative purposes.

9.5 INSTRUMENTATION

Basically, all gas chromatographs (GLC or GSC) consist of six basic components (Fig.9.5.1). They are

1. a carrier gas which is maintained at a high pressure is delivered to the instrument at a rapid and reproducible rate
2. a sample injection system
3. a separation column
4. one or more detectors
5. thermostatic chambers for the temperature regulation of the column and detectors,
6. an amplification and recorder system

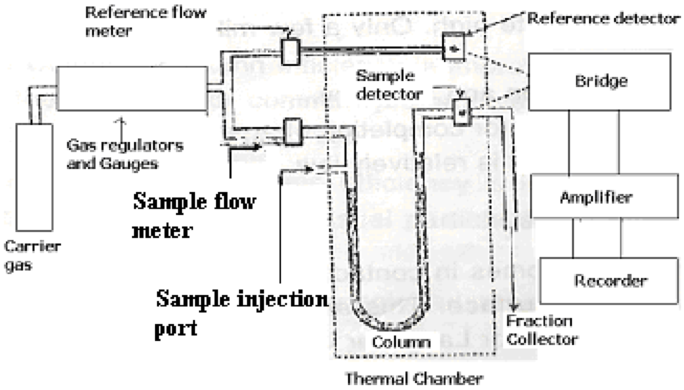


Figure 9.5.1: Schematic representation of GC

The sequence of gas chromatographic separation is as follows. The sample mixture is injected into the heating block where it is immediately vaporized. The vapours are carried by the carrier gas into the column inlet. The components are adsorbed at the head of the column by the stationary phase and then desorbed by fresh carrier gas. Depending on the distribution ratio, each component will travel at different rate through the column and give rise to the resolution of the bands. Finally, they will enter the detector attached to the end of the column and register a series of signals as functions of their concentration changes and rates of elution. Separation of binary mixture is shown in Figure 9.5.2.

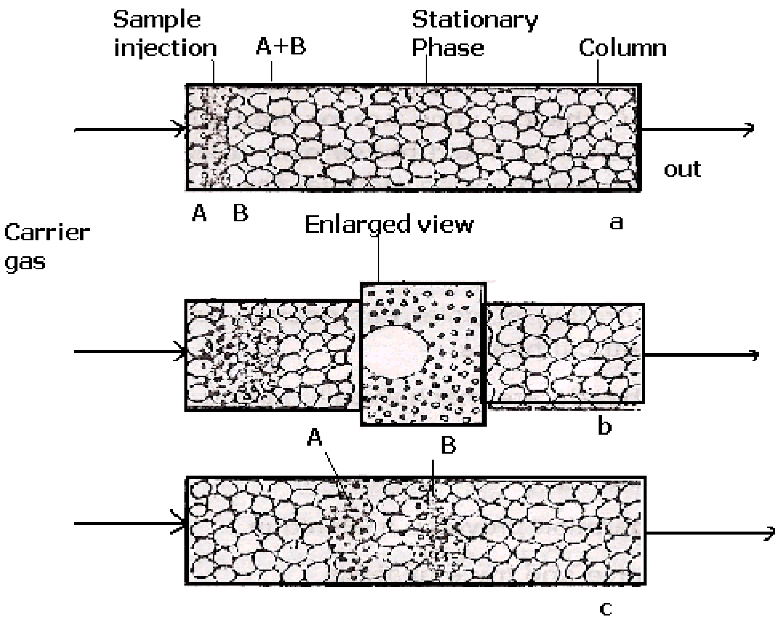


Figure 9.5.2: Separation of a binary mixture (A+B) Sample introduction; (b) Partial separation achieved; (c) Complete separation achieved.

9.5.1 Carrier Gas

The carrier gas is helium, nitrogen, hydrogen, argon or air which is usually determined by the detector being used. Availability, purity, consumption cost and type of detector determine the choice of the carrier gas. When analysing gas samples, the carrier is sometimes selected based on the sample's matrix. As a result of helium becoming more scarce, hydrogen is often being substituted for helium as a carrier gas in several applications. The purity of the carrier gas is also frequently determined by the detector, though the level of sensitivity needed can also play a significant role.

Typically, purities of 99.995% or higher are used. The most common purity grades required by modern instruments for the majority of sensitivities are 5.0 grades, or 99.999% pure meaning that there is a total of 10 ppm of impurities in the carrier gas that could affect the results. The highest purity grades in common use are 6.0 grades, but the need for detection at very low levels in some forensic and environmental applications has driven the need for carrier gases at 7.0 grade purity and these are now commercially available.

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9.5.2 Sample injection system

Liquid samples are introduced by hypodermic syringe through a self – sealing silicone rubber septum into a inlet chamber, which may be heated to give flash evaporation. Solid samples are dissolved in volatile liquids for introduction or may be introduced directly if they can be liquefied. Gaseous samples required special gas sampling valves for introduction into the carrier gas stream. Common injector types are:

- S/SL (split/split less) injector; a sample is introduced into a heated small chamber via a syringe through a septum – the heat facilitates volatilization of the sample and sample matrix. The carrier gas then either sweeps the entirety (split less mode) or a portion (split mode) of the sample into the column.
- On-column inlet; the sample is here introduced directly into the column in its entirety without heat, or at a temperature below the boiling point of the solvent. The low temperature condenses the sample into a narrow zone.
- PTV injector; Temperature-programmed sample introduction was first described by Vogt in 1979. Poy developed the programmed temperature vaporising injector; PTV by introducing the sample at a low initial liner temperature many of the disadvantages of the classic hot injection techniques could be circumvented.
- Gas source inlet or gas switching valve; gaseous samples in collection bottles are connected to what is most commonly a six-port switching valve. The carrier gas flow is not interrupted while a sample can be expanded into a previously evacuated sample loop. Upon switching, the contents of the sample loop are inserted into the carrier gas stream.
- P/T (Purge-and-Trap) system; An inert gas is bubbled through an aqueous sample causing insoluble volatile chemicals to be purged from the matrix. The volatiles are 'trapped' on an absorbent column (known as a trap or concentrator) at ambient temperature.

9.5.3 Column

The column can be made of glass or metal tubing and of any length from a few centimetres to over a hundred meters and can be coiled, bent or straight. The choice of column depends on the sample and the active measured. The main chemical attribute regarded when choosing a column is the polarity of the mixture, but functional groups can play a large part in column selection. The polarity of the sample must closely match the polarity of the column stationary phase to increase resolution and separation while reducing run time. The separation and run time also depend on the film thickness (of the stationary phase), the column diameter and the column length. There are three types of analytical columns are given below.

(i) Packed column
(ii) Open tubular column
(iii) Support coated tubular column

9.5.4 Detectors

Almost all the detectors monitor the GLC column effluent by measuring the changes in the composition arising from the variations in the eluted component. When a carrier gas alone is passing, they give zero signal. When a component is eluted, it is detected and it gives a signal proportional to the concentration of the component that is produced. The most commonly used detectors are the flame ionization detector (FID) and the thermal conductivity detector (TCD). Both are sensitive to a wide range of components, and both work over a wide range of concentrations. While TCDs are essentially universal and can be used to detect any component other than the carrier gas (as long as their thermal conductivities are different from that of the carrier gas, at detector temperature), FIDs are sensitive primarily to hydrocarbons, and are more sensitive to them than TCD. However, a FID cannot detect water. Both detectors are also quite robust. Since TCD is non-destructive, it can be operated in-series before a FID (destructive), thus providing complementary detection of the same analytes.

Other commercially available detectors are;

- ❖ Alkali flame detector (AFD) or alkali flame ionization detector (AFID)
- ❖ Flame photometric detector (FPD)
- ❖ Electron Capture Detector (ECD)
- ❖ Nitrogen–phosphorus detector (NPD)
- ❖ Dry electrolytic conductivity detector (DELCD)
- ❖ Hall electrolytic conductivity detector (ElCD)
- ❖ Helium ionization detector (HID)
- ❖ Infrared detector (IRD)
- ❖ Photo-ionization detector (PID)
- ❖ Pulsed discharge ionization detector (PDD)
- ❖ Thermionic ionization detector (TID)

9.5.5 Substrates

The solid support coated with high boiling liquid is known as the substrate. It acts as stationary phase in GLC. Good solvent property, differential partitioning of the component, low vapor pressure and high thermal stability are the general requirements for the liquid phase. Ex: polyglycols, paraffin oils, silicone oils and dodecyl phthalate.

9.5.6 Temperature control

A temperature programming is used for the controlled increase of temperature even during an analysis. The temperature programming may be carried out by natural, linear or matrix mode.

In general, the liner temperature programme is more common. The requirement for good temperature programming is given below.

a) Dual column system.
b) Separate heaters for injector, column, oven and detector system.
c) Differential flow controllers.
d) Low mass column oven for rapid heat transfer

e) Thin walled columns

f) Low liquid phase loading
g) Pure dry carrier gas.
h) Stable and non-bleeding injection systems.

9.6 Advantages of GC

1. This technique has a strong separation power and even complex mixture can be resolved into constituents.
2. The sensitivity is quite high. Only a few milligrams of the sample are sufficient for analysis.
3. It gives good precision and accuracy short time is required for completion of analysis
4. The cost of instruments relatively low

9.7 Applications of GC

1. The principal applications of GC are the qualitative and quantitative analysis of liquids, gases, vapors, particularly of organic compounds.
2. The detection of steroid drugs used by athletes and steroids administrated to animals in traces are being carried out by GLC
3. Pollutants such as formaldehyde, carbon monoxide, trichloroethylene, benzene and acrylonitrile can be monitored by GLC
4. GCL is frequently used in analysis of food
5. Dairy products are analyzed by GLC for aldehydes, acetones, fatty acids and milk sugars.
6. Though HPLC playing important role in drug analysis, GLC also finds valid application in drug analysis. Ex: analysis of commercial drug, blood, urine samples etc.,
7. Pyrolysis GC is use for the separation and identification of volatile materials like plastics, natural and synthetic polymers, paints and microbiological samples.
8. Inorganic compounds are analyzed by GC by derivatizations. A number of metals chelates have been separated by GLC.
9. Application of GC for environmental studies include separation and identification of polycyclic aromatic hydrocarbons, chlorinated pesticides, organophosphorus and Sulphur compounds, phenols, amines, organic acids etc.,
10. Gas chromatography is used extensively in forensic science. Disciplines as diverse as solid drug dose (pre-consumption form) identification and quantification, arson investigation, paint chip analysis, and toxicology cases, employ GC to identify and quantify various biological specimens and crime-scene evidence.

Check Your Progress – 4

1. What are the types of Gas chromatography?
2. Name some commercially available detectors?
3. What is known as substrates?
4. List some applications of GC?

9.8 ANSWER TO CHECK YOUR PROGRESS QUESTION

1. There are two types of chromatography. They are Gas liquid chromatography (GLC) and Gas solid chromatography (GSC)
2. Alkali flame detector (AFD)

   Flame photometric detector (FPD)

   Electron Capture Detector (ECD)
3. The solid support coated with high boiling liquid is known as the substrate. It acts as stationary phase in GLC
4. GCL is frequently used in analysis of food.
5. Dairy products are analyzed by GLC for aldehydes, acetones, fatty acids and milk sugars.
6. Gas chromatography is used extensively in forensic science.

9.9 SUMMARY

Gas Chromatography (GC) works on the principle of separation of compounds due to the difference in the partition behaviour between mobile phase and stationary phase. The components are separated according to the partition co-efficient and so no two components have same partition co-efficient for fixed combination of stationary phase and mobile phase. There are two types of GC such as Gas solid chromatography (GSC) and Gas Liquid Chromatography (GLC). The stationary phases in GSC and GLC differs slightly which exhibits different types of mechanism. It has a strong separation power and even complex mixture can be resolved into constituents and it gives good precision and accuracy short time is required for completion of analysis. It is normally used in food analysis, separation and identification of volatile materials like plastics, polymers etc.

9.10 KEY WORDS

- ❖ Carrier gas
- ❖ injector
- ❖ Detectors
- ❖ Substrate

9.11 SELF-ASSESSMENT QUESTION AND EXERCISES

1. Explain the principle of GC?
2. Discuss about the role of carrier gas?
3. What are the requirement needed for the good temperature programming?
4. what are the advantages of GC?

9.12 FURTHER READINGS

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10. GAS CHROMATOGRAPHIC –MASS SPECTRAL TECHNIQUE

Structure

10.1 INTRODUCTION

Gas chromatography mass spectrometry (GC/MS) is an instrumental technique, comprising a gas chromatograph (GC) coupled to a mass spectrometer (MS), to identify and quantify different mixture of chemicals. There are many advantages to using GC/MS for compound analysis, including its ability to separate complex mixtures, to assess analytes, and to determine trace levels of contamination in organic chemicals. This makes it ideal for the analysis of the hundreds of relatively low molecular weight compounds found in environmental materials. In order for a compound to be analysed by GC/MS it must be sufficiently volatile and thermally stable. It is widely used for chemical analysis, and especially for drug and environmental contamination testing. When combined with MS, GC/MS can be used in both full scan MS or select ion monitoring (SIM).

10.2 OBJECTIVES

After going through this unit, you will be able

- ❖ To gain knowledge on Gas chromatographic- Mass spectral techniques
- ❖ To know the principle of GC/MS involved in the technique.
- ❖ To develop knowledge on the GC/MS instrumentation and know the advantages of GC/MS.
- ❖ To know the applications of GC/MS techniques

10.3 PRINCIPLE

When two separate techniques such as gas chromatography (GC) and mass spectrometry (MS) are successfully combined to form gas chromatography mass spectrometry (GC-MS), the advantages become obvious. GC can separate many volatile and semi-volatile compounds but not always selectively detect them whereas MS can selectively detect many compounds but not always separate them. GC-MS is an ideal technique for qualitative and quantitative determination of volatile and semi-volatile organic compounds in a wide variety of samples. A detection limit as low as sub-nanogram is possible. The sample must be in solution for injection into the GC. This can simply be dissolution in a solvent, typically dichloromethane or extraction (with a Soxhlet, which is our most widely used method for extracting hydrocarbons form soil).

10.4 INSTRUMENTATION

10.4.1 Major components

The GC-MS is composed of two major building blocks (Figure 10.4.1): the gas chromatograph and the mass spectrometer. The gas chromatograph utilizes a capillary column which depends on the column's dimensions (length, diameter, film thickness) as well as the phase properties (e.g. 5% phenyl polysiloxane). The difference in the chemical properties between different molecules in a mixture and their relative affinity for the stationary phase of the column will promote separation of the molecules as the sample travels the length of the column. The molecules are retained by the column and then elute (come off) from the column at different times (called the retention time), and this allows the mass spectrometer downstream to capture, ionize, accelerate, deflect, and detect the ionized molecules separately. The mass spectrometer does this by breaking each molecule into ionized fragments and detecting these fragments using their mass-to-charge ratio.

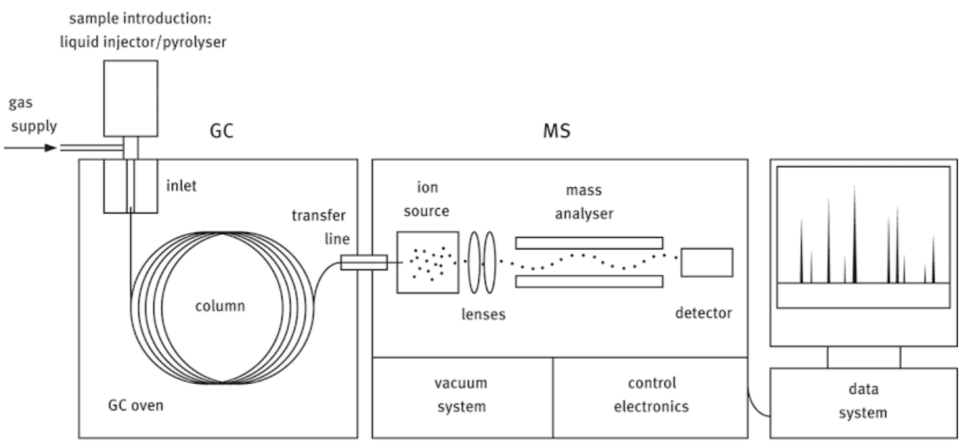


Figure 10.4.1 Schematic representation of GC MS

Gas Chromatographic – Mass Spectral Technique

NOTES

These two components, used together, allow a much finer degree of substance identification than either unit used separately. It is not possible to make an accurate identification of a particular molecule by gas chromatography or mass spectrometry alone. The mass spectrometry process normally requires a very pure sample while gas chromatography using a traditional detector (e.g. Flame ionization detector) cannot differentiate between multiple molecules that happen to take the same amount of time to travel through the column (i.e. have the same retention time), which results in two or more molecules that co-elute. Sometimes two different molecules can also have a similar pattern of ionized fragments in a mass spectrometer (mass spectrum). Combining the two processes reduces the possibility of error, as it is extremely unlikely that two different molecules will behave in the same way in both a gas chromatograph and a mass spectrometer. Therefore, when an identifying mass spectrum appears at a characteristic retention time in a GC-MS analysis, it typically increases certainty that the analyte of interest is in the sample.

The most common type of mass spectrometer (MS) associated with a gas chromatograph (GC) is the quadrupole mass spectrometer, sometimes referred to by the Hewlett-Packard (now Agilent) trade name "Mass Selective Detector" (MSD). Another relatively common detector is the ion trap mass spectrometer. Additionally, one may find a magnetic sector mass spectrometer, however these particular instruments are expensive and bulky and not typically found in high-throughput service laboratories. Other detectors may be encountered such as time of flight (TOF), tandem quadrupoles (MS-MS) (see below), or in the case of an ion trap MS^n where n indicates the number mass spectrometry stages.

10.4.2 Ionization

After the molecules travel the length of the column, pass through the transfer line and enter into the mass spectrometer they are ionized by various methods with typically only one method being used at any given time. Once the sample is fragmented it will then be detected, usually by an electron multiplier, which essentially turns the ionized mass fragment into an electrical signal that is then detected. The ionization technique chosen is independent of using full scan or SIM.

a) Electron ionization

By far the most common and perhaps standard form of ionization is electron ionization (EI). The molecules enter into the MS (the source is a quadrupole or the ion trap itself in an ion trap MS) where they are bombarded with free electrons emitted from a filament, not unlike the filament one would find in a standard light bulb. The electrons bombard the molecules, causing the molecule to fragment in a characteristic and reproducible way. This "hard ionization" technique results in the creation of more fragments of low mass-to-charge ratio (m/z) and few, if any, molecules approaching the molecular mass unit. Hard ionization is considered by mass spectrometrists as the employ of molecular electron bombardment, whereas "soft ionization" is charge by molecular collision with an introduced gas. The molecular fragmentation pattern is dependent

upon the electron energy applied to the system, typically 70 eV (electron Volts).

b) *Cold electron ionization*

The "hard ionization" process of electron ionization can be softened by the cooling of the molecules before their ionization, resulting in mass spectra that are richer in information. In this method named cold electron ionization (cold-EI) the molecules exit the GC column, mixed with added helium make up gas and expand into vacuum through a specially designed supersonic nozzle, forming a supersonic molecular beam (SMB). Collisions with the make-up gas at the expanding supersonic jet reduce the internal vibrational (and rotational) energy of the analyte molecules, hence reducing the degree of fragmentation caused by the electrons during the ionization process. Cold-EI mass spectra are characterized by an abundant molecular ion while the usual fragmentation pattern is retained, thus making cold-EI mass spectra compatible with library search identification techniques. The enhanced molecular ions increase the identification probabilities of both known and unknown compounds, amplify isomer mass spectral effects and enable the use of isotope abundance analysis for the elucidation of elemental formulae.

c) *Chemical ionization*

In chemical ionization a reagent gas, typically methane or ammonia is introduced into the mass spectrometer. Depending on the technique (positive CI or negative CI) chosen, this reagent gas will interact with the electrons and analyte and cause a 'soft' ionization of the molecule of interest. A softer ionization fragments the molecule to a lower degree than the hard ionization of EI. One of the main benefits of using chemical ionization is that a mass fragment closely corresponding to the molecular weight of the analyte of interest is produced. In positive chemical ionization (PCI) the reagent gas interacts with the target molecule, most often with a proton exchange. This produces the species in relatively high amounts. In negative chemical ionization (NCI) the reagent gas decreases the impact of the free electrons on the target analyte. This decreased energy typically leaves the fragment in great supply.

10.5 ANALYSIS

A mass spectrometer is typically utilized in one of two ways: full scan or selective ion monitoring (SIM). The typical GC-MS instrument is capable of performing both functions either individually or concomitantly, depending on the setup of the particular instrument. The primary goal of instrument analysis is to quantify an amount of substance. This is done by comparing the relative concentrations among the atomic masses in the generated spectrum. Two kinds of analysis are possible, comparative and original.

Comparative analysis essentially compares the given spectrum to a spectrum library to see if its characteristics are present for some sample in the library. This is best performed by a computer because there are a myriad of visual distortions that can take place due to variations in scale. Computers can also simultaneously correlate more data (such as the retention times identified by GC), to more accurately relate certain data. Deep learning was shown to lead to promising results in the identification of VOCs from raw GC-MS data.

NOTES

Gas
Chromatographic –
Mass Spectral
Technique

NOTES

Another method of analysis measures the peaks in relation to one another. In this method, the tallest peak is assigned 100% of the value, and the other peaks being assigned proportionate values. All values above 3% are assigned. The total mass of the unknown compound is normally indicated by the parent peak. The value of this parent peak can be used to fit with a chemical formula containing the various elements which are believed to be in the compound. The isotope pattern in the spectrum, which is unique for elements that have many natural isotopes, can also be used to identify the various elements present. Once a chemical formula has been matched to the spectrum, the molecular structure and bonding can be identified, and must be consistent with the characteristics recorded by GC-MS.

Typically, this identification is done automatically by programs which come with the instrument, given a list of the elements which could be present in the sample. A "full spectrum" analysis considers all the "peaks" within a spectrum. Conversely, selective ion monitoring (SIM) only monitors selected ions associated with a specific substance. This is done on the assumption that at a given retention time, a set of ions is characteristic of a certain compound. This is a fast and efficient analysis, especially if the analyst has previous information about a sample or is only looking for a few specific substances. When the amount of information collected about the ions in a given gas chromatographic peak decreases, the sensitivity of the analysis increases. So, SIM analysis allows for a smaller quantity of a compound to be detected and measured, but the degree of certainty about the identity of that compound is reduced.

10.5.1 Full scan MS

When collecting data in the full scan mode, a target range of mass fragments is determined and put into the instrument's method. An example of a typical broad range of mass fragments to monitor would be m/z 50 to m/z 400. The determination of what range to use is largely dictated by what one anticipates being in the sample while being cognizant of the solvent and other possible interferences. A MS should not be set to look for mass fragments too low or else one may detect air (found as m/z 28 due to nitrogen), carbon dioxide (m/z 44) or other possible interference. Additionally if one is to use a large scan range then sensitivity of the instrument is decreased due to performing fewer scans per second since each scan will have to detect a wide range of mass fragments.

Full scan is useful in determining unknown compounds in a sample. It provides more information than SIM when it comes to confirming or resolving compounds in a sample. During instrument method development it may be common to first analyze test solutions in full scan mode to determine the retention time and the mass fragment fingerprint before moving to a SIM instrument method.

10.5.2 Selective ion monitoring

In selective ion monitoring (SIM) certain ion fragments are entered into the instrument method and only those mass fragments are detected by the mass spectrometer. The advantages of SIM are that the detection limit is lower since the instrument is only looking at a small number of fragments (e.g. three fragments) during each scan. More scans can take

place each second. Since only a few mass fragments of interest are being monitored,

matrix interferences are typically lower. To additionally confirm the likelihood of a potentially positive result, it is relatively important to be sure that the ion ratios of the various mass fragments are comparable to a known reference standard.

10.6 INTERPRETATION OF GC-MS

In general, the level of attraction during stationary phase causes components to separate and elute at different times, which shows as peaks on the resulting chromatogram.

10.6.1 The X-Axis: Retention Time

Usually, the x-axis of the gas chromatogram shows the amount of time taken for the analytes to pass through the column and reach the mass spectrometer detector. The peaks that are shown correspond to the time at which each of the components reached the detector. The type of column used during the analysis, as well as the GC parameters (e.g. flow rate, injection temperature, oven temperature, etc.), have a large impact on the retention time. As a result, when comparing retention times from different analyses or different labs, it's critical that the same parameters are used to ensure accuracy.

10.6.2 The Y-Axis: Concentration or Intensity Counts

Typically, the y-axis, or the area of the peak, is a reflection of the amount of a specific analyte that's present. When looking at a GC/MS chromatogram, the area will be based on the number of counts taken by the mass spectrometer detector at the point of retention. However, it's important to note that some compounds will have a better affinity with the detector and the peaks will appear larger than the actual concentration would be in relation to the other peaks on the chromatogram, which we often see in compounds that ionize readily. To overcome this challenge, our experts run standards with known concentrations of compounds to ensure accurate counts. In addition, unknown compounds are identified based on their retention times of known standards with other detectors. The mass spectrometer detector then allows identification of a compound by mass spectrum obtained at the time of testing.

10.6.3 Differences in Gas Chromatogram Models

At a high level, depending on the type of sample and the desired outcome, gas chromatography analysis can use several different means of sample introduction, such as static headspace analysis, thermal desorption, and direct injection, as well as different types of detectors, such as flame ionization (FID), electron capture (ECD), and—of course—mass spectrometry. As a result, chromatograms can often differ in how they appear. However, even with variations, the basics of understanding chromatograms, as outlined above, remain the same. But one key point we'd like to reiterate is that it's important to remember that the systems and parameters used for an analysis need to be similar when comparing results from two or more different analyses. This ensures you're getting the most accurate comparison and drawing meaningful insights.

10.6.4 GC/MS Chromatogram Examples

Epoxy Outgassing

An epoxy used in a device was failing to function as intended in Figure 10.5.4.1. After performing epoxy outgassing analysis, it was tested and compared with two different samples: a reference epoxy (top) and the failed epoxy (bottom).

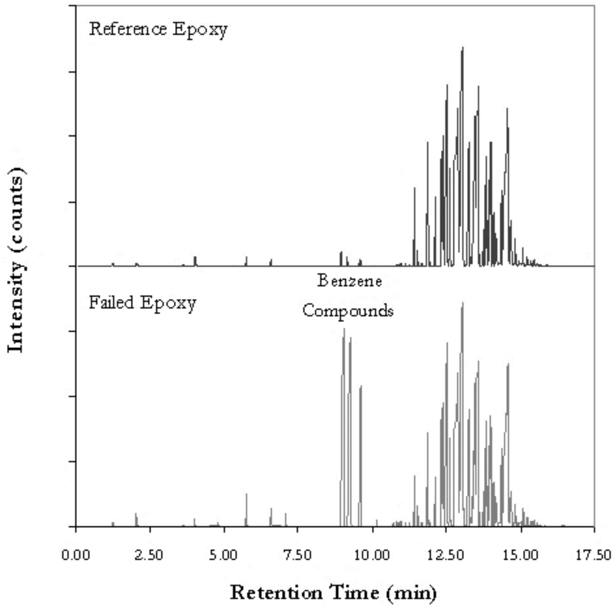


Figure 10.5.4.1 GC/MS testing results for epoxy outgassing analysis

Results showed that the failed epoxy has a large cluster of Benzene compounds (y-axis) at approximately the 9-minute mark on the retention timeline (x-axis)—which is something that wasn't seen in the reference epoxy.

Vegetable Oil Analysis

As shown in Figure 10.5.4.2, a food manufacturer was considering switching to a new vegetable cooking oil.

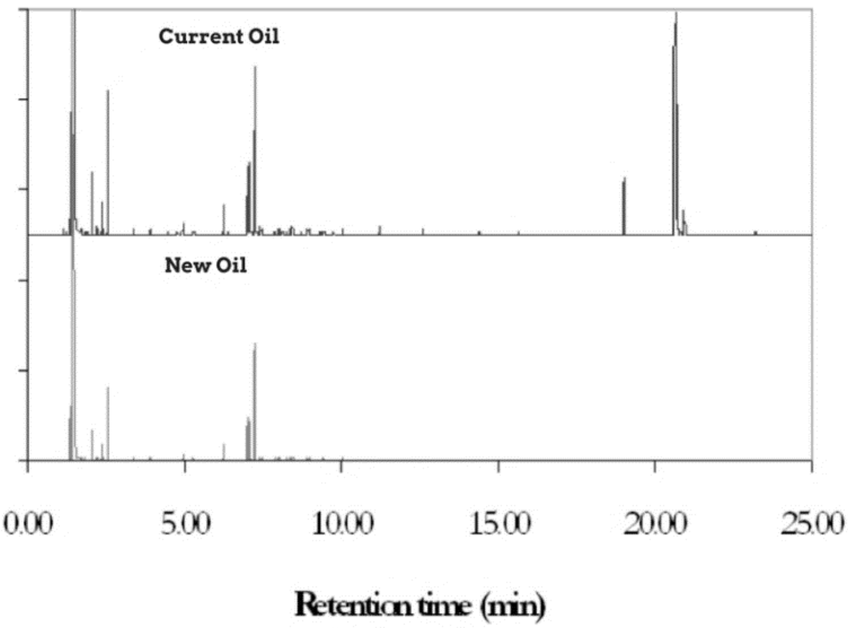


Figure 10.5.4.2 Example for GC/MS testing of vegetable oil

After a vegetable oil analysis of both current and a potentially new oil, it was found that the current oil contained several different compounds including: palmitic acid (retention time of 19.00 minutes), linoleic acid (retention time of 20.6 minutes), oleic acid (retention time of 20.7 minutes), stearic acid (retention time of 20.9 minutes).

Residual Solvent Analysis

This analysis was performed in order to know if ethanol was used in the manufacturing process and was still present in the final product.

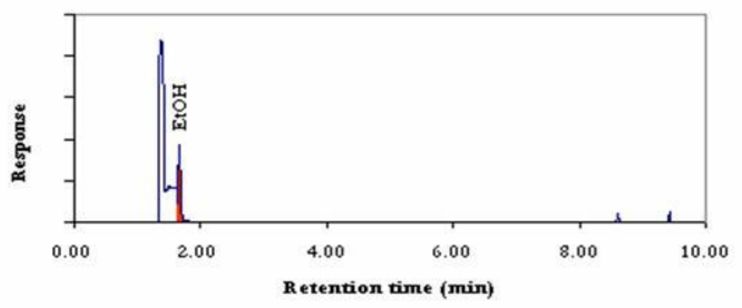


Figure 10.5.4.3 Example for residual solvent analysis

Using GC/MS headspace analysis, residual solvent testing was conducted as shown in Figure 10.5.4.3 a possible residual solvent peak was found at approximately 1.67 minutes. Then the findings are compared to ethanol mass spectrum data to confirm the residual peak for the presence of ethanol.

10.7. APPLICATIONS OF GC-MS

1. GC-MS is mainly used in the determination of organic compounds. It is also becoming the tool of choice for tracking organic pollutants in the environment, which has contributed to its increased adoption in environmental studies.
2. GC-MS can analyze the particles from a human body in order to help link a criminal to a crime. The analysis of fire debris using GC-MS is well established, and there is even an established American Society for Testing and Materials (ASTM) standard for fire debris analysis. GCMS/MS is especially useful here as samples often contain very complex matrices and results, used in court, need to be highly accurate.
3. GC-MS is increasingly used for detection of illegal narcotics, and may eventually supplant drug-sniffing dogs. GC-MS is also commonly used in forensic toxicology to find drugs and/or poisons in biological specimens of suspects, victims, or the deceased.
4. GC-MS is the main tool used in sports anti-doping laboratories to test athletes' urine samples for prohibited performance enhancing drugs.
5. GC-MS is used for the analysis of unknown organic compound mixtures. One critical use of this technology is the use of GC-MS to determine the composition of bio-oils processed from raw biomass.

6. Foods and beverages contain numerous aromatic compounds, some naturally present in the raw materials and some forming during processing. GC-MS is extensively used for the analysis of these compounds which include esters, fatty acids, alcohols, aldehydes, terpenes etc. It is also used to detect and measure contaminants from spoilage or adulteration which may be harmful and which is often controlled by governmental agencies, for example pesticides.
7. Dozens of congenital metabolic diseases also known as inborn errors of metabolism (IEM) are now detectable by newborn screening tests, especially the testing using gas chromatography–mass spectrometry. GC-MS can determine compounds in urine even in minor concentration. These compounds are normally not present but appear in individuals suffering with metabolic disorders.
8. In combination with isotopic labeling of metabolic compounds, the GC-MS is used for determining metabolic activity. Most applications are based on the use of ^{13}C as the labeling and the measurement of ^{13}C-^{12}C ratios with an isotope ratio massspectrometer (IRMS); an MS with a detector designed to m e a s u r e a

Check Your Progress – 4

1. What is the principle of Gas chromatographic-mass spectral technique?
2. What are the types of ionization technique?
3. Discuss about the advantages of selective ion monitoring (SIM)
4. What is meant by residual solvent analysis?

few select ions and return values as ratios.

10.8 ANSWER TO CHECK YOUR PROGRESS QUESTION

1. Gas chromatography (GC) and mass spectrometry (MS) are successfully combined to form gas chromatography mass spectrometry (GC-MS). GC can separate many volatile and semi-volatile compounds but not always selectively detect them whereas MS can selectively detect many compounds but not always separate them
2. There are three types of ionization they are Electron ionization, cold-electron ionization and chemical ionization.
3. The advantages of SIM are that the detection limit is lower since the instrument is only looking at a small number of fragments (e.g. three fragments) during each scan. More scans can take place each second. Since only a few mass fragments of interest are being monitored, matrix interferences are typically lower.

4. Residual solvent analysis is a technique performed to know if ethanol was used in the manufacturing process and was still present in the final product.

10.9 SUMMARY

The Gas Chromatography/Mass Spectrometry (GC/MS) instrument separates chemical mixtures and identifies the components at a molecular level. the Separation takes place through GC and identification through MS. It is one of the most accurate tools for analyzing environmental samples. The GC works on the principle that a mixture will separate into individual substances when heated. The heated gases are carried through a column with an inert gas (such as helium). As the separated substances emerge from the column opening, they flow into the MS. Mass spectrometry identifies compounds by the mass of the analyte molecule and it is considered as the only definitive analytical detector.

10.10 KEY WORDS

- ❖ Mass spectrometer
- ❖ Ionization
- ❖ Retention time
- ❖ Solvent analysis

10.11 SELF-ASSESSMENT QUESTION AND EXERCISES

1. Briefly explain n the instrumentation of GC-MS?
2. Discuss about the various types of ionization.
3. What is known as Retention time?
4. Write some applications for GC-MS?

10.12 FURTHER READINGS

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11. HIGH PRESSURE LIQUID CHROMATOGRAPHY

Structure

11.1 INTRODUCTION

High-performance liquid chromatography (HPLC) formerly known as high-pressure liquid chromatography is a technique in analytical chemistry used to separate, identify, and quantify each component in a mixture. It relies on pumps to pass a pressurized liquid solvent containing the sample mixture through a column filled with a solid adsorbent material. Each component in the sample interacts slightly differently with the adsorbent material, causing different flow rates for the different components and leading to the separation of the components as they flow out of the column. Following on the seminal work of Martin and Synge in 1941, it was predicted by Cal Giddings, Josef Huber, and others in the 1960s that LC could be operated in the high-efficiency mode by reducing the packing-particle diameter substantially below the typical LC (and GC) level of 150 μm and using pressure to increase the mobile phase velocity. These predictions underwent extensive experimentation and refinement throughout the 60s into the 70s. Early developmental research began to improve LC particles, and the invention of Zipax, a superficially porous particle, was promising for HPLC technology.

11.2 OBJECTIVES

After going through this unit, you will be able

- ❖ To know the general principle and types of HPLC
- ❖ To know in detail about Normal and reversed phase liquid chromatography
- ❖ To learn about the instrumentation involved in HPLC.
- ❖ To visit the applications of HPLC

11.3. PRINCIPLE

It is the fact that the resolving power of a chromatographic column increases with column length and the number of theoretical plates per unit length. Since the number of theoretical plates is related to the surface area of the stationary phase, better resolution will be achieved if the particle size is small. The smaller the particle size is the greater the resistance to eluant flow. So the flow rates achieved are relatively low and this gives greater time for band broadening. Faster flow rate is not possible because it damages the matrix structure of the stationary phase.

To rectify these difficulties, the technology has been improved with the new particle size stationary phase which can withstand these pressures of pumping systems that can give reliable flow rates. These developments have occurred in adsorption, partition, ion-exchange, exclusion and affinity chromatography and resulted in faster and better resolution and explain why HPLC has emerged as powerful and versatile form of chromatography.

All the factors affecting separation on liquid column chromatography are applied to this technology also [example: plate height, sample distribution between the stationary and mobile phase, selection of the stationary and mobile phases]

11.4 TYPES OF HPLC

- ❖ Partition chromatography
- ❖ Normal–phase chromatography
- ❖ Displacement chromatography
- ❖ Reversed-phase chromatography (RPC)
- ❖ Size-exclusion chromatography
- ❖ Ion-exchange chromatography
- ❖ Bio affinity chromatography
- ❖ Aqueous normal-phase chromatography

11.5 NORMAL–PHASE CHROMATOGRAPHY

Normal–phase chromatography was one of the first kinds of HPLC that chemists developed. Also known as normal-phase HPLC (NP-HPLC) this method separates analytes based on their affinity for a polar stationary surface such as silica, hence it is based on analyte ability to engage in polar interactions (such as hydrogen-bonding or dipole-dipole type of interactions) with the sorbent surface. NP-HPLC uses a non-polar, non-aqueous mobile phase (e.g., Chloroform), and works effectively for separating analytes readily soluble in non-polar solvents. The analyte associates with and is retained by the polar stationary phase. Adsorption strengths increase with increased analyte polarity. The interaction strength depends not only on the functional groups present in the structure of the analyte molecule, but also on steric factors. The effect of steric hindrance on interaction strength allows this method to resolve (separate) structural isomers.

The use of more polar solvents in the mobile phase will decrease the retention time of analytes, whereas more hydrophobic solvents tend to induce slower elution (increased retention times). Very polar solvents such as traces of water in the mobile phase tend to adsorb to the solid surface of the stationary phase forming a stationary bound (water) layer which is considered to play an active role in retention. This behavior is somewhat peculiar to normal phase chromatography because it is governed almost exclusively by an adsorptive mechanism (i.e., analytes interact with a solid surface rather than with the solvated layer of a ligand attached to the sorbent surface; see also reversed-phase HPLC below). Adsorption chromatography is still widely used for structural isomer separations in both column and thin-layer chromatography formats on activated (dried) silica or alumina supports.

Partition- and NP-HPLC fell out of favor in the 1970s with the development of reversed-phase HPLC because of poor reproducibility of retention times due to the presence of a water or protic organic solvent layer on the surface of the silica or alumina chromatographic media. This layer changes with any changes in the composition of the mobile phase (e.g., moisture level) causing drifting retention times. Recently, partition chromatography has become popular again with the development of Hilic bonded phases which demonstrate improved reproducibility, and due to a better understanding of the range of usefulness of the technique.

11.6. REVERSED PHASE HPLC (RP-HPLC)

11.6.1 Stationary phase

RP-HPLC has a non-polar stationary phase and an aqueous, moderately polar mobile phase. One common stationary phase is a silica which has been surface-modified with RMe_2SiCl, where R is a straight chain alkyl group such as $C_{18}H_{37}$ or C_8H_{17}. With such stationary phases, retention time is longer for molecules which are less polar, while polar molecules elute more readily (early in the analysis). RP-HPLC is so commonly used that it is often incorrectly referred to as "HPLC" without further specification. The pharmaceutical industry regularly employs RP-HPLC to qualify drugs before their release.

RP-HPLC operates on the principle of hydrophobic interactions, which originates from the high symmetry in the dipolar water structure and plays the most important role in all processes in life science. RP-HPLC allows the measurement of these interactive forces. The binding of the analyte to the stationary phase is proportional to the contact surface area around the non-polar segment of the analyte molecule upon association with the ligand on the stationary phase. This solvo-phobic effect is dominated by the force of water for "cavity-reduction" around the analyte and the C18-chain versus the complex of both.

Structural properties of the analyte molecule play an important role in its retention characteristics. In general, an analyte with a larger hydrophobic surface area (C–H, C–C, and generally non-polar atomic bonds, such as S-S and others) is retained longer because it is non-interacting with the water structure. On the other hand, analytes with higher polar surface area (conferred by the presence of polar groups, such as -OH, $-NH_2$, COO^- or $-NH^{3+}$ in their structure) are less retained as they are better integrated into water. Such interactions are subject to steric effects in that very large molecules may have only restricted access to the pores of the stationary phase, where the interactions with surface ligands (alkyl chains) take place. Such surface hindrance typically results in less retention.

Retention time increases with hydrophobic (non-polar) surface area. Branched chain compounds elute more rapidly than their corresponding linear isomers because the overall surface area is decreased. Similarly, organic compounds with single C–C bonds elute later than those with a C=C or C–C triple bond, as the double or triple bond is shorter than a single C–C bond. Another important factor is the mobile phase pH since it can change the hydrophobic character of the analyte. For this reason, most methods use a buffering agent, such as sodium phosphate, to control the pH. Buffers serve multiple purposes: control of pH, neutralize the charge on the silica surface of the stationary phase and act as ion pairing agents to neutralize analyte charge.

11.6.2Mobile phases

Mixtures of water or aqueous buffers and organic solvents are used to elute analytes from a reversed-phase column. The solvents must be miscible with water, and the most common organic solvents used are acetonitrile, methanol, and tetrahydrofuran (THF). Other solvents can be used such as ethanol or 2-propanol (isopropyl alcohol). Elution can be performed isocratically (the water-solvent composition does not change during the separation process) or by using a solution gradient (the water-solvent composition changes during the separation process, usually by decreasing the polarity). The pH of the mobile phase can have an important role on the retention of an analyte and can change the selectivity of certain analytes. Charged analytes can be separated on a reversed-phase column by the use of ion-pairing (also called ion-interaction). This technique is known as reversed-phase ion-pairing chromatography.

11.7. INSTRUMENTATION

A schematic diagram of typical HPLC system is show in Figure 11.4.1. which consists of

1. solvent reservoir and mixing system
2. high pressure pump
3. sample inlet pump
4. a column and
5. a detector and recording unit

The suitable solvents (mobile liquid phase) from the reservoir are allowed to enter the mixing chamber where a homogenous mixture is

obtained. A pump which is maintaining high pressures draw the solvent from the mixing chambers and pushes it through the column.

The sample is injected into the high-pressure liquid carrier stream between the pump and the column. The separation takes place on the column. The flow rates are 1-2 ml/min with pressure unto several thousand Psi. The effluent passes through the detector and is collected for the further studies.

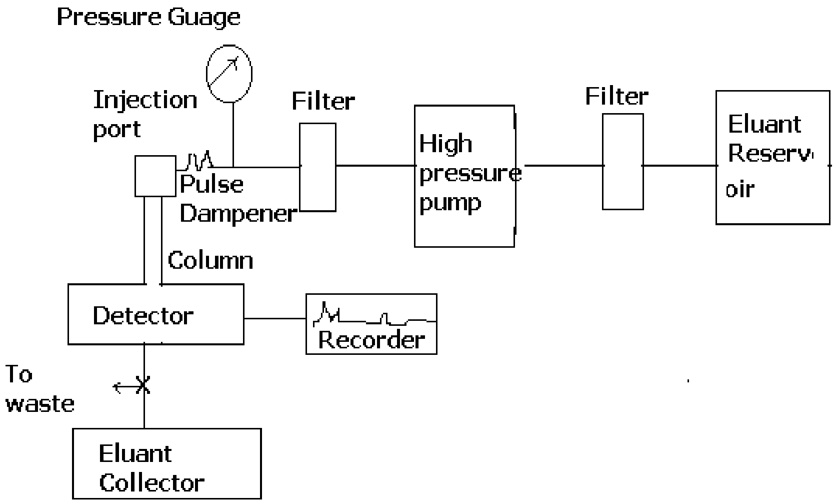


Figure 11.4.1: Schematic diagram of HPLC

11.7.1 Column

The column used are generally made of stainless steel in such a way that they have to withstand for the pressures upto 5.5X 10^{7}Pa. Generally straight columns of 20 to 50 cm in length and 1 to 4mm in diameter are used. There are porous plugs of stainless steel or Teflon in the ends of the column to retain the packing material. In some analysis the column temperature is controlled thermostatically. An HPLC typically includes two columns: an analytical column responsible for the separation and a guard column. The guard column is placed before the analytical column, protecting it from contamination.

Analytical columns

The most common type of HPLC column is a stainless-steel tube with an internal diameter between 2.1 mm and 4.6 mm and a length between 30 mm and 300 mm. The column is packed with 3–10 μm porous silica particles with either an irregular or a spherical shape. Typical column efficiencies are 40 000–60 000 theoretical plates/m. Assuming a V /V of approximately 50, a 25-cm column with 50 000 plates/m has 12 500 theoretical plates and a peak capacity of 110. Capillary columns use less solvent and, because the sample is diluted to a lesser extent, produce larger signals at the detector. These columns are made from fused silica capillaries with internal diameters from 44–200 μm and lengths of 50–250 mm.

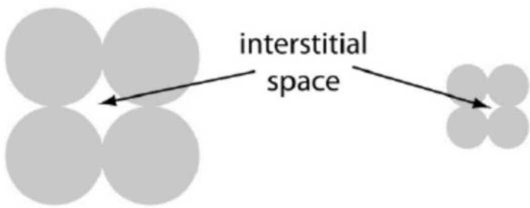


Figure 11.4.2 The packing of smaller particles creates smaller interstitial spaces than the packing of larger particles.

Capillary columns packed with 3–5 μm particles have been prepared with column efficiencies of up to 250 000 theoretical plates. One limitation to a packed capillary column is the back pressure that develops when trying to move the mobile phase through the small interstitial spaces between the particulate micron-sized packing material (Figure 11.4.2). Because the tubing and fittings that carry the mobile phase have pressure limits, a higher back pressure requires a lower flow rate and a longer analysis time. Monolithic columns, in which the solid support is a single, porous rod, offer column efficiencies equivalent to a packed capillary column while allowing for faster flow rates.

Guard columns

Two problems tend to shorten the lifetime of an analytical column. First, solutes binding irreversibly to the stationary phase degrade the column's performance by decreasing the available stationary phase. Second, particulate material injected with the sample may clog the analytical column. To minimize these problems, we place a guard column before the analytical column. Guard columns usually contain the same particulate packing material and stationary phase as the analytical column, but are significantly shorter and less expensive. Because they are intended to be sacrificial, guard columns are replaced regularly.

Packing of column

Three forms of column packing material are available. They are

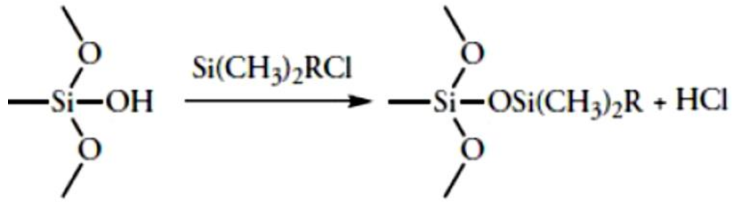
1. Microporous supports-where microporous ramify through the particles which are 5 to 10 μm in diameter
2. Pellicular supports – where the porous particles are coated onto an inert solid core such as glass bead of 40 μm in diameter, and
3. Bonded phases – where the stationary phase is chemically bonded to an inert support.

Column Packing Procedure

Commercial columns are available in the market. The most widely used technique for column packing is the high pressure slurring technique. A suspension of packing material with solvent is prepared. The slurry is pumped rapidly at high pressure onto column with porous plug at its outlet. The resulting packed column then is prepared for use by running the developing solvent through the column and hence the packing is equilibrated with developing solvent.

11.7.2 Stationary phases

In liquid–liquid chromatography the stationary phase is a liquid film coated on a packing material, typically 3–10 μm porous silica particles. Because the stationary phase may be partially soluble in the mobile phase, it may elute, or bleed from the column over time. To prevent the loss of stationary phase, which shortens the column's lifetime, it is covalently bound to the silica particles. Bonded stationary phases are created by reacting the silica particles with an organochlorosilane of the general form $Si(CH_3)_2RCl$, where R is an alkyl, or substituted alkyl group.



To prevent unwanted interactions between the solutes and any remaining –SiOH groups, $Si(CH_3)_3Cl$ is added, converting the unreacted sites to $–SiOSi(CH_3)_3$; such columns are designated as end-capped. The properties of a stationary phase depend on the organosilane's alkyl group. If R is a polar functional group, then the stationary phase is polar. Examples of polar stationary phases include those where R contains a cyano ($–C_2H_4CN$), a diol ($–C_3H_6OCH_2CHOHCH_2OH$), or an amino ($–C_3H_6NH_2$) functional group. Because the stationary phase is polar, the mobile phase is a nonpolar or moderately polar solvent. The combination of a polar stationary phase and a nonpolar mobile phase is called normal-phase chromatography.

In reversed-phase chromatography, which is the more common form of HPLC, the stationary phase is nonpolar and the mobile phase is polar. The most common nonpolar stationary phases use an organochlorosilane where the R group is an n-octyl (C_8) or n-octyldecyl (C_{18}) hydrocarbon chain. Most reversed-phase separations are carried out using a buffered aqueous solution as a polar mobile phase, or with other polar solvents, such as methanol and acetonitrile. Because the silica substrate may undergo hydrolysis in basic solutions, the pH of the mobile phase must be less than 7.5.

11.7.3 Mobile phase

The choice of mobile phase depends on the type of the separation. Isocratic separations involve a single solvent or two or more solvents mixed in fixed proportion. A gradient elution system may also be used by the use of suitable gradient programmer, where the composition of the developing solvent is continuously changed. All the solvents used for HPLC must be specially purified, since traces of impurities can affect the column. All the solvents are degassed before use, otherwise gassing can alter column resolution and interfere with the monitoring of column effluent. Degassing may be carried out by warming the solvents, by stirring it vigorously with the magnetic stirrer or other methods.

The elution order of solutes in HPLC is governed by polarity. For a normal-phase separation, solutes of lower polarity spend proportionally less time in the polar stationary phase and are the first solutes to elute from the column. Given a particular stationary phase, retention times in normal-phase HPLC are controlled by adjusting the mobile phase's properties. For example, if the resolution between two solutes is poor, switching to a less polar mobile phase keeps the solutes on the column for a longer time and provides more opportunity for their separation. In reversed-phase HPLC the order of elution is the opposite of that in a normal-phase separation, with more polar solutes eluting first. Increasing the polarity of the mobile phase leads to longer retention times.

Shorter retention times require a mobile phase of lower polarity. Choosing a mobile phase depends on the need which has following criteria;

- Using the polarity index
- Adjusting selectivity
- Isocratic and gradient elution.

11.7.4 Pumping systems

The main feature of the pumping system is that it is capable of delivering an output of at least 3.4×10^7 Pa and there must be pulses less flow through the system. The flow delivery must be at least 10 cm^3/min for normal analysis and up to 30 cm^3/min for preparative analysis. All materials in the pump should be chemically inert to all solvents. Various pumping systems are available on the principle of constant pressure or constant displacement.

Constant pressure pump produces a pulseless flow through the column, but any decrease in the permeability of the column will result in lower flow rate for which the pump will not compensate.

Constant displacement pumps maintain constant flow rate through the column irrespective of changing conditions within the column. All constant displacement pumps have in built safety cut-out mechanisms, so that if the pressure within the chromatographic systems changes from pre-set limits, the pump is inactivated

11.7.5 Detector Systems

The sensitivity of the detector system should be sufficiently high and stable since the quantity of a sample used frequently is very small. Most commonly used detector is an available wavelength or spectrophotometer, fluorimeter, refractive index monitor or an electrochemical detector.

Spectroscopic detectors

The most popular HPLC detectors take advantage of an analyte's UV/Vis absorption spectrum. These detectors range from simple designs, in which the analytical wavelength is selected using appropriate filters, to a modified spectrophotometer in which the sample compartment includes a flow cell. Figure 11.5.5.1 shows the design of a typical flow cell for a detector using a diode array spectrometer. The flow cell has a volume of 1–10 μL and a path length of 0.2–1 cm. When using a UV/Vis detector the resulting chromatogram is a plot of absorbance as a function of elution time. If the detector is a diode array spectrometer, then we also can display the result as a three-dimensional chromatogram showing absorbance as a function of wavelength and elution time. One limitation to using absorbance is that the mobile phase cannot absorb at the wavelengths we wish to monitor. Absorbance detectors provide detection limits of as little as 100 pg^{-1} ng of injected analyte.

High Pressure Liquid Chromatography

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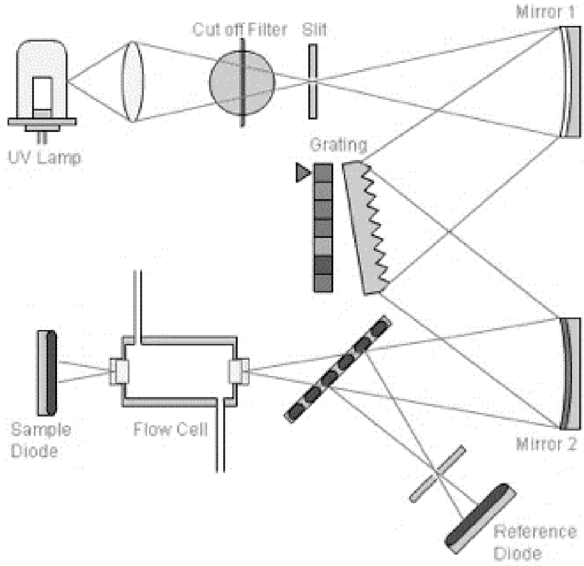


Figure 11.5.5.1 Schematic diagram showing spectroscopic detectors Electrochemical detectors

Another common group of HPLC detectors are those based on electrochemical measurements such as amperometry, voltammetry, coulometry, and conductivity. Figure 11.5.5.2, for example, shows an amperometric flow cell.

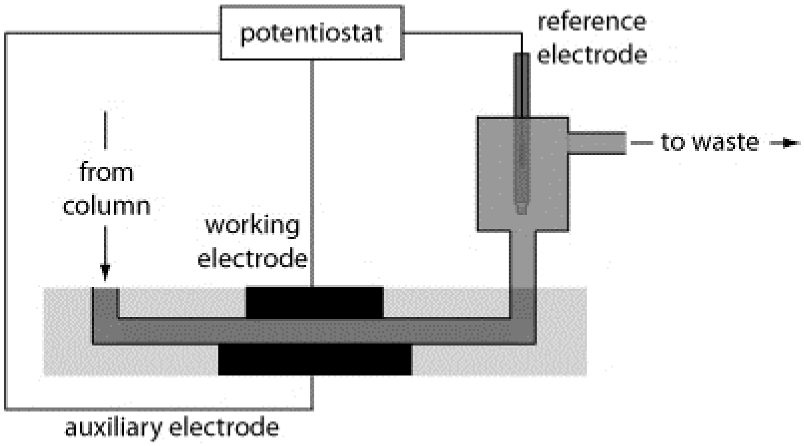


Figure 11.5.5.2: Schematic diagram showing a flow cell for an electrochemical detector

Effluent from the column passes over the working electrode, which is held at a constant potential, relative to a downstream reference electrode that completely oxidizes or reduces the analytes. The current flowing between the working electrode and the auxiliary electrode serves as the analytical signal. Detection limits for amperometric electrochemical detection are from 10 pg^{-1} ng of injected analyte.

11.8. Experimental Procedure

The sample is introduced as an infinitely narrow band into the column. There are two methods for the injection of the sample. In the first method, specially designed micro syringe which withstands high pressure has been used. The sample is injected through a septum in an injection port, directly onto the column packing or onto a small plug of inert material immediately above the column packing.

This can be done while the system is under pressure, or when the pump is turned off the pressure has dropped to near atmospheric, then the injection is made and the pump is switched on again. This is termed as stop flow injection. The second method makes use of loop injector. A metal loop of small volume can be filled with the sample. By using suitable value, the eluant from the pump is passed through the loop, the outlet of which leads directly onto the column. Automatic versions of loop injectors are commercially available.

11.9 APPLICATIONS OF HPLC

- HPLC has many applications in both laboratory and clinical science. It is a common technique used in pharmaceutical development, as it is a dependable way to obtain and ensure product purity. In manufacturing HPLC can produce extremely high quality (pure) products, it is not always the primary method used in the production of bulk drug materials.
- HPLC is also used for detection of illicit drugs in urine. The most common method of drug detection is an immunoassay. HPLC has been used to detect a variety of agents like doping agents, drug metabolites, glucuronide conjugates, amphetamines, opioids, cocaine, BZDs, ketamine, LSD, cannabis, and pesticides.
- HPLC is obviously useful in observing multiple species in collected samples, as well, but requires the use of standard solutions when information about species identity is sought out. It is used as a method to confirm results of synthesis reactions, as purity is essential in this type of research. However, mass spectrometry is still the more reliable way to identify species.
- Medical use of HPLC can include drug analysis, but falls more closely under the category of nutrient analysis. While urine is the most common medium for analyzing drug concentrations, blood serum is the sample collected for most medical analyses with HPLC.

Check Your Progress – 4

1. State different types of HPLC
2. Explain packing of column.
3. Give some uses of HPLC?

11.10 ANSWER TO CHECK YOUR PROGRESS QUESTION

1. Partition chromatography
   Normal–phase chromatography
   Displacement chromatography
   Reversed-phase chromatography (RPC)
   Size-exclusion chromatography
   Ion-exchange chromatography

2. There are 3 methods of packing of column
   - ❖ Microporous supports-where microporous ramify through the particles which are 5 to 10 μm in diameter
   - ❖ Pellicular supports – where the porous particles are coated onto an inert solid core such as glass bead of 40 μm in diameter, and
   - ❖ Bonded phases – where the stationary phase is chemically bonded to an inert support.
3. It is used in the pharmaceutical development, as it is a dependable way to obtain and ensure product purity.

HPLC is also used for detection of illicit drugs in urine.

11.11 SUMMARY

High-performance liquid chromatography (HPLC) is an analytical technique used for the separation, identification, and quantification of individual compound in a mixture. Separation process involves two phase of compounds such as mobile phase and stationary phase. There are various types of HPLC used for the advance method of purification of chemicals. HPLC is highly used in laboratory and clinical science for the purification of drugs, chemical compounds etc. Use of HPLC strongly suggest the high purity of the material.

11.12 KEY WORDS

- ❖ Mass spectrometer
- ❖ Effluent
- ❖ Column packing

11.13 SELF-ASSESSMENT QUESTION AND EXERCISES

1. Briefly describe the instrumentation setup for HPLC?
2. write the procedure for column packing.

11.14 FURTHER READINGS

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BLOCK-IV
ION CHROMATOGRAPHY, PURIFICATION TECHNIQUES AND IONIC SEPARATION

Unit 12: ION CHROMATOGRAPHY

Structure

12.1 INTRODUCTION

Ion Chromatography is a method of separating ions based on their distinct retention rates in a given solid phase packing material. Given different retention rates for two anions or two cations, the elution time of each ion will differ, allowing for detection and separation of one ion before the other. Detection methods are separated between electrochemical methods and spectroscopic methods. This guide will cover the principles of retention rates for anions and cations, as well as describing the various types of solid-state packing materials and eluents that can be used.

12.2 OBJECTIVES

After going through this unit, you will be able to

- ❖ know the definition and theory behind ion chromatography
- ❖ know the diverse applications of ion chromatography
- ❖ get idea on gel permeation chromatography and its application

12.3 Theory of ion chromatography

Retention models in anion chromatography

The retention model for anionic chromatography can be split into two distinct models, one for describing eluents with a single anion, and the other for describing eluents with complexing agents present. Given an eluent anion or an analyte anion, two phases are observed, the stationary phase (denoted by S) and the mobile phase (denoted by M). As such, there is equilibrium between the two phases for both the eluent anions and the analyte anions that can be described by the following equation.

$$y * [A_M^{x-}] + x * [E_S^{y-}] \Leftrightarrow y * [A_S^{x-}] + x * [E_M^{y-}]$$

This yields an equilibrium constant as given in following equation,

$$K_{A,E} = \frac{[A_S^{x-}]^y [E_M^{y-}]^x \gamma^y_{A_S^{x-}} \gamma^x_{E_S^{y-}}}{[A_M^{x-}]^y [E_S^{y-}]^x \gamma^y_{A_M^{x-}} \gamma^x_{E_S^{y-}}}$$

Given the activity of the two ions cannot be found in the stationary or mobile phases, the activity coefficients are set to 1. Two new quantities are then introduced. The first is the distribution coefficient, D, which is the ratio of analyte concentrations in the stationary phase to the mobile phase by,

$$D_A = \frac{[A_S]}{[A_M]}$$

The second is the retention factor, k, which is the distribution coefficient times the ratio of volume between the two phases,

$$k_A^1 = D_A * \frac{V_S}{V_M}$$

Substituting the above two quantities, the equilibrium constant can be written as

$$K_{A,E} = (k_A^1 \frac{V_M}{V_S})^y * (\frac{[E_M^{y-}]}{[E_S^{y-}]})^x$$

Given there is usually a large difference in concentrations between the eluent and the analyte (with magnitudes of 10 greater eluent), equation 4 can be re-written under the assumption that all the solid phase packing material's functional groups are taken up by E. As such, the stationary E can be substituted with the exchange capacity divided by the charge of E. This yields

$$K_{A,E} = (k_A^1 \frac{V_M}{V_S})^y * (\frac{Q}{\gamma})^{-x} [E_M^{y-}]$$

Solving for the retention factor, the following equation is developed.

$$z * [A_M^{x-}] + x * [B_S^{z-}] \Leftrightarrow z * [A_S^{x-}] + x * [B_M^{z-}]$$

The equilibrium between the two analytes is determined as

$$K_{A,B} = \frac{[A_S^{x-}]^z [B_M^{z-}]^x}{[A_M^{x-}]^z [B_S^{z-}]^x}$$

Which shows the relationship between retention factor and parameters like eluent concentration and the exchange capacity, which allows parameters of the ion chromatography to be manipulated and the retention factors to be determined. The equilibrium constant (ignoring activity) can be written as:

$$\alpha_{A,B} = \frac{[A_S^{x-}][B_M^{z-}]}{[A_M^{x-}][B_S^{z-}]}$$

This equation only works for a single analyte present, but a relationship for the selectivity between two analytes [A] and [B] can easily be determined. This can then be simplified into a logarithmic form as the following two equations:

$$log\alpha_{A,B} = \frac{1}{z}logK_{A,B} + \frac{x-z}{z}log\frac{k_A^1 V_M}{V_S}$$

$$log\alpha_{A,B} = \frac{1}{x}logK_{A,B} + \frac{x-z}{z}log\frac{k_A^1 V_M}{V_S}$$

When the two charges are the same, it can be seen that the selectivity is only a factor of the selectivity coefficients and the charges. When the two charges are different, it can be seen that the two retention factors are dependent upon each other. In situations with a polyatomic eluent, three models are used to account for the multiple anions in the eluent. The first is the dominant equilibrium model, in which one anion is so dominant in concentration; the other eluent anions are ignored. The dominant equilibrium model works best for multivalence analytes. The second is the effective charge model, where an effective charge of the eluent anions is found, and a relationship similar to EQ is found with the effective charge. The effective charge models works best with monovalent analytes. The third is the multiple eluent species model, where the equation below describes the retention factor:

$$logK_A^1 = C_3 - (\frac{X_1}{a} + \frac{X_2}{b} + \frac{X_3}{c}) - logC_P$$

C_3 is a constant that includes the phase volume ratio between stationary, the equilibrium constant, and mobile and the exchange capacity. C_p is the total concentration of the eluent species. X_1, X_2, X_3, correspond to the shares of a particular eluent anion in the retention of the analyte.

Retention models of cation chromatography

For eluents with a single cation and analytes that are alkaline earth metals, heavy metals or transition metals, a complexing agent is used to bind with the metal during chromatography. This introduces the quantity A(m) to the retention rate calculations, where A(m) is the ratio of free metal ion to the total concentration of metal. Following a similar derivation to the single anion case,

Ion
Chromatography

NOTES

$$K_{A,E} = (\frac{k_A^1}{\alpha_M \phi})^y * (\frac{Q}{\gamma})^{-x} [E_M^{y+}]^x$$

Which after solving the retention factor becomes,

$$k_A^1 = \alpha_M \phi * K_{A,E}^{\frac{1}{\gamma}} (\frac{Q}{\gamma})^{\frac{x}{y}} ([E_M^{y+}]^{-\frac{x}{y}}$$

From this expression, the retention rate of the cation can be determined from eluent concentration and the ratio of free metal ions to the total concentration of the metal, which itself is depended on the equilibrium of the metal ion with the complexing agent.

Solid phase packing materials

The solid phase packing material used in the chromatography column is important to the exchange capacity of the anion or cation. There are many types of packing material, but all share a functional group that can bind either the anion or the cation complex. The functional group is mounted on a polymer surface or sphere, allowing large surface area for interaction.

Packing material for anion chromatography

The primary functional group used for anion chromatography is the ammonium group. Amine groups are mounted on the polymer surface, and the pH is lowered to produce ammonium groups. As such, the exchange capacity is depended on the pH of the eluent. To reduce the pH dependency, the protons on the ammonium are successively replaced with alkyl groups until the all the protons are replaced and the functional group is still positively charged, but pH independent. Trimethylamine (NMe) and dimethylanolamine are the two packing materials used in most of the anion chromatography.

Packing material for cation chromatography

Cation chromatography allows for the use of both organic polymer based and silica gel based packing material. In the silica gel based packing material, the most common packing material is a polymer-coated silica gel. The silicate is coated in polymer, which is held together by cross-linking of the polymer. Polybutadiene maleic acid is then used to create a weakly acidic material, allowing the analyte to diffuse through the polymer and exchange. Silica gel based packing material is limited by the pH dependent solubility of the silica gel and the pH dependent linking of the silica gel and the functionalized polymer. However, silica gel based packing material is suitable for separation of alkali metals and alkali earth metals.

Organic polymer based packing material is not limited by pH like the silica gel materials are, but are not suitable for separation of alkali metals and alkali earth metals. The most common functional group is the sulfonic acid group attached with a spacer between the polymer and the sulfonic acid group.

Detection Methods

Spectroscopic detection methods

Photometric detection in the UV region of the spectrum is a common method of detection in ion chromatography. Photometric methods limit the eluent possibilities, as the analyte must have a unique absorbance wavelength to be detectable. Cations that do not have a unique absorbance wavelength, i.e. the eluent and other contaminants have similar UV visible spectra can be complexed to for UV visible compounds. This allows detection of the cation without interference from eluents. Coupling the chromatography with various types of spectroscopy such as Mass spectroscopy or IR spectroscopy can be a useful method of detection. Inductively coupled plasma atomic emission spectroscopy is a commonly used method.

Direct conductivity methods

Direct conductivity methods take advantage of the change in conductivity that an analyte produces in the eluent, which can be modeled by

$$\Delta K = \frac{(\Lambda_A - \Lambda_g) * C_s}{1000}$$

where equivalent conductivity is defined as,

$$\Lambda = \frac{L}{A * R} * \frac{1}{C}$$

With L being the distance between two electrodes of area A and R being the resistance the ion creates. C is the concentration of the ion. The values of Equivalent conductivity of the analyte and of the eluent common ions are given in Table 12.1. The conductivity can be plotted over time, and the peaks that appear represent different ions coming through the column as described by

$$K_{peak} = (\Lambda_A - \Lambda_g) * C_A$$

Table 12.1. Equivalent conductivity of the analyte and of the eluent common ions

Cations	Λ^+ (S cm^2 eq^{-1})	Anions	Λ^+ (S cm^2 eq^{-1})
H^+	350	OH^-	198
Li^+	39	F^-	54
Na^+	50	Cl^-	76
K^+	74	Br	78
NH^{4+}	73	I^-	77
½ Mg^{2+}	53	NO_2^-	72
½ Ca^{2+}	60	NO_3^-	71
½ Sr^{2+}	59	HCO_3^-	45
½ Ba^{2+}	64	½ CO_3^{2-}	72
½ Zn^{2+}	52	$H_2PO_4^-$	33
½ Hg^{2+}	53	½ HPO_4^-	57
½ Cu^{2+}	55	⅓ PO_4^-	69
½ Pb^{2+}	71	½ SO_4^{2-}	80
½ Co^{2+}	53	CN^-	82
⅓ Fe^{3+}	70	SCN^-	66
$N(Et)^{4+}$	33	*Acetate*	41
		½ *Phthalate*	38
		Propionate	36
		Benzoate	32
		Salicylate	30
		½ *Oxalate*	74

Eluents

The choice of eluent depends on many factors, namely, pH, buffer capacity, the concentration of the eluent, and the nature of the eluent's reaction with the column and the packing material.

Eluents in anion chromatography

In non-suppressed anion chromatography, where the eluent and analyte are not altered between the column and the detector, there is a wide range of eluents to be used. In the non-suppressed case, the only issue that could arise is if the eluent impaired the detection ability (absorbing in a similar place in a UV-spectra as the analyte for instance). As such, there are a number of commonly used eluents. Aromatic carboxylic acids are used in conductivity detection because of their low self-conductivity.

Aliphatic carboxylic acids are used for UV/visible detection because they are UV transparent. Inorganic acids can only be used in photometric detection. In suppressed anion chromatography, where the eluent and analyte are treated between the column and detection, fewer eluents can be used. The suppressor modifies the eluent and the analyte, reducing the self-conductivity of the eluent and possibly increasing the self-conductivity of the analyte. Only alkali hydroxides and carbonates, borates, hydrogen carbonates, and amino acids can be used as eluents.

Eluents in cation chromatography

The primary eluents used in cation chromatography of alkali metals and ammoniums are mineral acids such as HNO_3. When the cation is multivalent, organic bases such as ethylenediamine serve as the main eluents. If both alkali metals and alkali earth metals are present, hydrochloric acid or 2,3-diaminopropionic acid is used in combination with a pH variation. If the chromatography is unsuppressed, the direct conductivity measurement of the analyte will show up as a negative peak due to the high conductivity of the H^+ in the eluent, but simple inversion of the data can be used to rectify this discrepancy. If transition metals or H^+ are the analytes in question, complexing carboxylic acids are used to suppress the charge of the analyte and to create photometrically detectable complexes, forgoing the need for direct conductivity as the detection method.

Ion Exchange Chromatography

Theory

Ion-exchange chromatography (IEC) is a chromatography process that separates ions and polar molecules based on their affinity to the ion exchanger. It works on almost any kind of charged molecule including large proteins, small nucleotides, and amino acids. However, ion chromatography must be done in conditions that are one unit away from the isoelectric point of a protein. Ion-exchange chromatography separates molecules based on their respective charged groups. Ion exchange chromatography retains analyte molecules on the column based on coulombic (ionic) interactions. The ion exchange chromatography matrix consists of positively and negatively charged ions. Essentially, molecules undergo electrostatic interactions with opposite charges on the stationary phase matrix. The stationary phase consists of an immobile matrix that contains charged ionizable functional groups or ligands. The stationary phase surface displays ionic functional groups (R-X) that interact with analyte ions of opposite charge.

To achieve electroneutrality, these inert charges couple with exchangeable counterions in the solution. Ionizable molecules that are to be purified compete with these exchangeable counterions for binding to the immobilized charges on the stationary phase. These ionizable molecules are retained or eluted based on their charge. Initially, molecules that do not bind or bind weakly to the stationary phase are first to wash away. Altered conditions are needed for the elution of the molecules that bind to the stationary phase. The concentration of the exchangeable counterions, which competes with the molecules for binding, can be increased or the pH can be changed.

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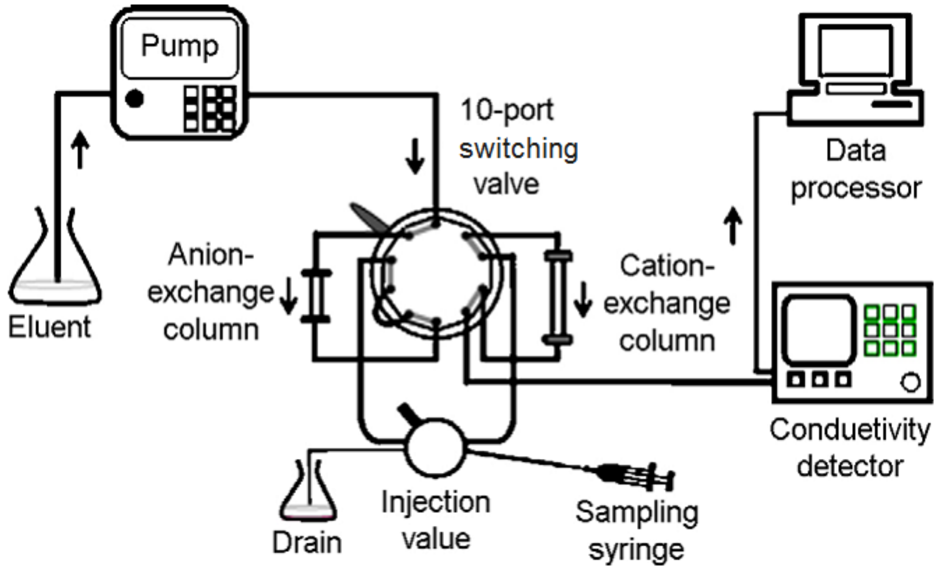


Figure: Schematic representation of IEC

A change in pH affects the charge on the particular molecules and, therefore, alters binding. The molecules then start eluting out based on the changes in their charges from the adjustments. Further such adjustments can be used to release the protein of interest. Additionally, concentration of counterions can be gradually varied to separate ionized molecules. This type of elution is called gradient elution. On the other hand, step elution can be used in which the concentration of counterions are varied in one step. This type of chromatography is further subdivided into cation exchange chromatography and anion-exchange chromatography. Positively charged molecules bind to cation exchange resins while negatively charged molecules bind to anion exchange resins. The ionic compound consisting of the cationic species M+ and the anionic species B- can be retained by the stationary phase.

Cation exchange chromatography retains positively charged cations because the stationary phase displays a negatively charged functional group:

$$\text{R-X}^-\text{C}^+ + \text{M}^+\text{B}^- \rightleftarrows \text{R-X}^-\text{M}^+ + \text{C}^+ + \text{B}^-$$

Anion exchange chromatography retains anions using positively charged functional group:

$$\text{R-X}^+\text{A}^- + \text{M}^+\text{B}^- \rightleftarrows \text{R-X}^+\text{B}^- + \text{M}^+ + \text{A}^-$$

Note that the ion strength of either C+ or A- in the mobile phase can be adjusted to shift the equilibrium position, thus retention time.

Procedure

Before ion-exchange chromatography can be initiated, it must be equilibrated. The stationary phase must be equilibrated to certain requirements that depend on the experiment that you are working with. Once equilibrated, the charged ions in the stationary phase will be attached to its opposite charged exchangeable ions. Exchangeable ions such as Cl^- or Na^+. Next, a buffer should be chosen in which the desired protein can bind to. After equilibration, the column needs to be washed. The washing

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phase will help elute out all impurities that does not bind to the matrix while the protein

of interest remains bounded. This sample buffer needs to have the same pH as the buffer used for equilibration to help bind the desired proteins. Uncharged proteins will be eluted out of the column at a similar speed of the buffer flowing through the column.

Once the sample has been loaded onto to the column and the column has been washed with the buffer to elute out all non-desired proteins, elution is carried out to elute the desired proteins that are bound to the matrix. Bound proteins are eluted out by utilizing a gradient of linearly increasing salt concentration. With increasing ionic strength of the buffer, the salt ions will compete with the desired proteins in order to bind to charged groups on the surface of the medium. This will cause desired proteins to be eluted out of the column. Proteins that have a low net charge will be eluted out first as the salt concentration increases causing the ionic strength to increase.

Proteins with high net charge will need a higher ionic strength for them to be eluted out of the column. It is possible to perform ion exchange chromatography in bulk, on thin layers of medium such as glass or plastic plates coated with a layer of the desired stationary phase, or in chromatography columns. Thin layer chromatography or column chromatography share similarities in that they both act within the same governing principles; there is constant and frequent exchange of molecules as the mobile phase travels along the stationary phase. It is not imperative to add the sample in minute volumes as the predetermined conditions for the exchange column have been chosen so that there will be strong interaction between the mobile and stationary phases.

Furthermore, the mechanism of the elution process will cause a compartmentalization of the differing molecules based on their respective chemical characteristics. This phenomenon is due to an increase in salt concentrations at or near the top of the column, thereby displacing the molecules at that position, while molecules bound lower are released at a later point when the higher salt concentration reaches that area. These principles are the reasons that ion exchange chromatography is an excellent candidate for initial chromatography steps in a complex purification procedure as it can quickly yield small volumes of target molecules regardless of a greater starting volume.

Comparatively simple devices are often used to apply counterions of increasing gradient to a chromatography column. Counterions such as copper (II) are chosen most often for effectively separating peptides and amino acids through complex formation. Simple device can be used to create a salt gradient. Elution buffer is consistently being drawn from the chamber into the mixing chamber, thereby altering its buffer concentration. Generally, the buffer placed into the chamber is usually of high initial concentration, whereas the buffer placed into the stirred chamber is usually of low concentration. As the high concentration buffer from the left chamber is mixed and drawn into the column, the buffer concentration of the stirred column gradually increase. Altering the shapes of the stirred chamber, as well as of the limit buffer, allows for the production of concave, linear, or convex gradients of counterion. A multitude of different mediums are used for the stationary phase.

Among the most common immobilized charged groups used are trimethylaminoethyl (TAM), triethylaminoethyl (TEAE), diethyl-2-hydroxypropylaminoethyl (QAE), aminoethyl (AE), diethylaminoethyl (DEAE), sulpho (S), sulphomethyl (SM), sulphopropyl (SP), carboxy (C), and carboxymethyl (CM).

Successful packing of the column is an important aspect of ion chromatography. Stability and efficiency of a final column depends on packing methods, solvent used, and factors that affect mechanical properties of the column. In contrast to early inefficient dry- packing methods, wet slurry packing, in which particles that are suspended in an appropriate solvent are delivered into a column under pressure, shows significant improvement. Three different approaches can be employed in performing wet slurry packing: the balanced density method (solvent's density is about that of porous silica particles), the high viscosity method (a solvent of high viscosity is used), and the low viscosity slurry method (performed with low viscosity solvents). Polystyrene is used as a medium for ion- exchange. It is made from the polymerization of styrene with the use of divinylbenzene and benzoyl peroxide. Such exchangers form hydrophobic interactions with proteins which can be irreversible. Due to this property, polystyrene ion exchangers are not suitable for protein separation. They are used on the other hand for the separation of small molecules in amino acid separation and removal of salt from water. Polystyrene ion exchangers with large pores can be used for the separation of protein but must be coated with a hydrophilic substance.

Cellulose based medium can be used for the separation of large molecules as they contain large pores. Protein binding in this medium is high and has low hydrophobic character. DEAE is an anion exchange matrix that is produced from a positive side group of diethylaminoethyl bound to cellulose or Sephadex. Agarose gel based medium contain large pores as well but their substitution ability is lower in comparison to dextrans. The ability of the medium to swell in liquid is based on the cross-linking of these substances, the pH and the ion concentrations of the buffers used.

Incorporation of high temperature and pressure allows a significant increase in the efficiency of ion chromatography, along with a decrease in time. Temperature has an influence of selectivity due to its effects on retention properties. The retention factor ($k = (t_R^g - t_M^g)/(t_M^g - t_{ext})$) increases with temperature for small ions, and the opposite trend is observed for larger ions. Despite ion selectivity in different mediums, further research is being done to perform ion exchange chromatography through the range of 40–175 ^oC. An appropriate solvent can be chosen based on observations of how column particles behave in a solvent. Using an optical microscope, one can easily distinguish a desirable dispersed state of slurry from aggregated particles.

Applications

Ion exchange chromatography can be used to separate proteins because they contain charged functional groups. The ions of interest (in this case charged proteins) are exchanged for another ions (usually H^+) on a charged solid support. The solutes are most commonly in a liquid phase,

which tends to be water. Take for example proteins in water, which would be a liquid phase that is passed through a column.

A use of ion chromatography can be seen in the argentation ion chromatography. Usually, silver and compounds containing acetylenic and ethylenic bonds have very weak interactions. This phenomenon has been widely tested on olefin compounds. The ion complexes the olefins make with silver ions are weak and made based on the overlapping of pi, sigma, and d orbitals and available electrons therefore cause no real changes in the double bond. This behavior was manipulated to separate lipids, mainly fatty acids from mixtures in to fractions with differing number of double bonds using silver ions. The ion resins were impregnated with silver ions, which were then exposed to various acids (silicic acid) to elute fatty acids of different characteristics.

The main beneficial advantages are reliability, very good accuracy and precision, high selectivity, high speed, high separation efficiency, and low cost of consumables. The most significant development related to ion chromatography are new sample preparation methods; improving the speed and selectivity of analytes separation; lowering of limits of detection and limits of quantification; extending the scope of applications; development of new standard methods; miniaturization and extending the scope of the analysis of a new group of substances.

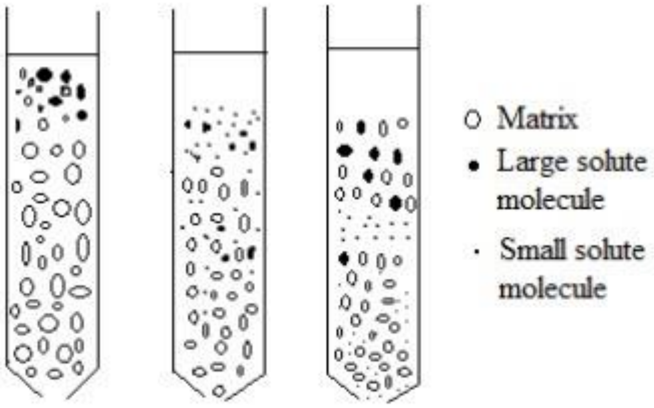
IC is used in different aspects of product development and quality control testing. Therefore, IC has been employed in drugs in the form of tablets and capsules in order to determine the amount of drug dissolve with time. IC is also widely used for detection and quantification of excipients or inactive ingredients used in pharmaceutical formulations. Detection of sugar and sugar alcohol in such formulations through IC has been done due to these polar groups getting resolved in ion column. IC methodology also established in analysis of impurities in drug substances and products.

12.4 GEL PERMEATION CHROMATOGRAPHY

Theory

Gel permeation chromatography (GPC) is a type of size exclusion chromatography (SEC), that separates analytes on the basis of size. The technique is often used for the analysis of polymers. As a technique, SEC was first developed in 1955 by Lathe and Ruthven. The term gel permeation chromatography can be traced back to J.C. Moore of the Dow Chemical Company who investigated the technique in 1964 and the proprietary column technology was licensed to Waters Corporation, who subsequently commercialized this technology in 1964. GPC systems and consumables are now also available from a number of manufacturers. It is often necessary to separate polymers, both to analyze them as well as to purify the desired product.

When characterizing polymers, it is important to consider the dispersity (Đ) as well the molecular weight. Polymers can be characterized by a variety of definitions for molecular weight including the number average molecular weight (M_n), the weight average molecular weight (M_w), the size average molecular weight (M_z), or the viscosity molecular weight (M_v). GPC allows for the determination of Đ as well as M_v and based on other data, the M_n, M_w, and M_z can be determined.



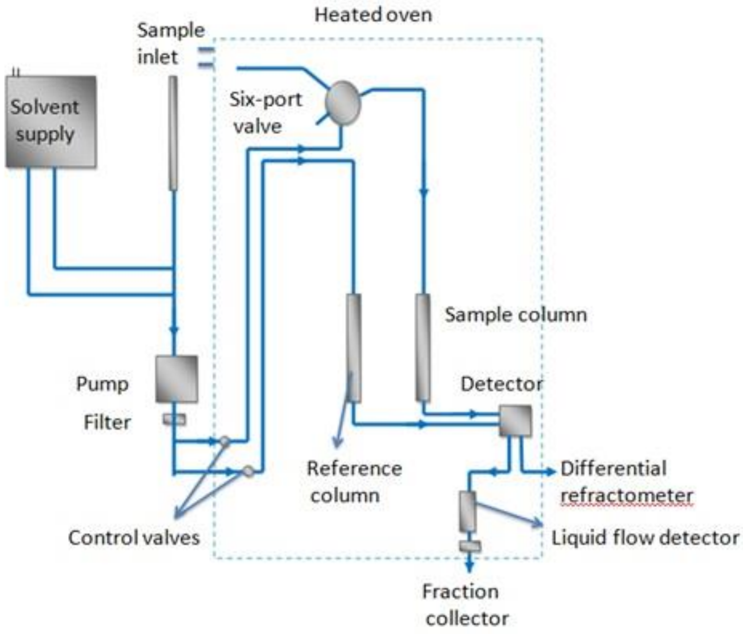
Principle of size exclusion chromatography

GPC separates based on the size or hydrodynamic volume (radius of gyration) of the analytes. This differs from other separation techniques which depend upon chemical or physical interactions to separate analytes. Separation occurs via the use of porous beads packed in a column. The smaller analytes can enter the pores more easily and therefore spend more time in these pores, increasing their retention time. These smaller molecules spend more time in the column and therefore will elute last. Conversely, larger analytes spend little if any time in the pores and are eluted quickly. All columns have a range of molecular weights that can be separated. If an analyte is too large, it will not be retained; conversely, if the analyte is too small, it may be retained completely. Analytes that are not retained are eluted with the free volume outside of the particles (V_o), while analytes that are completely retained are eluted with volume of solvent held in the pores (V_i). The total volume can be considered by the following equation, where V_g is the volume of the polymer gel and V_t is the total volume:

As can be inferred, there is a limited range of molecular weights that can be separated by each column, and therefore the size of the pores for the packing should be chosen according to the range of molecular weight of analytes to be separated. For polymer separations the pore sizes should be on the order of the polymers being analyzed. If a sample has a broad molecular weight range it may be necessary to use several GPC columns in tandem to fully resolve the sample.

Instrumentation

Gel permeation chromatography is conducted almost exclusively in chromatography columns. The experimental design is not much different from other techniques of liquid chromatography. Samples are dissolved in an appropriate solvent, in the case of GPC these tend to be organic solvents and after filtering the solution it is injected onto a column. The separation of multicomponent mixture takes place in the column. The constant supply of fresh eluent to the column is accomplished by the use of a pump. Since most analytes are not visible to the naked eye a detector is needed. Often multiple detectors are used to gain additional information about the polymer sample. The availability of a detector makes the fractionation convenient and accurate.



Schematic of gel permeation chromatography system

Gels

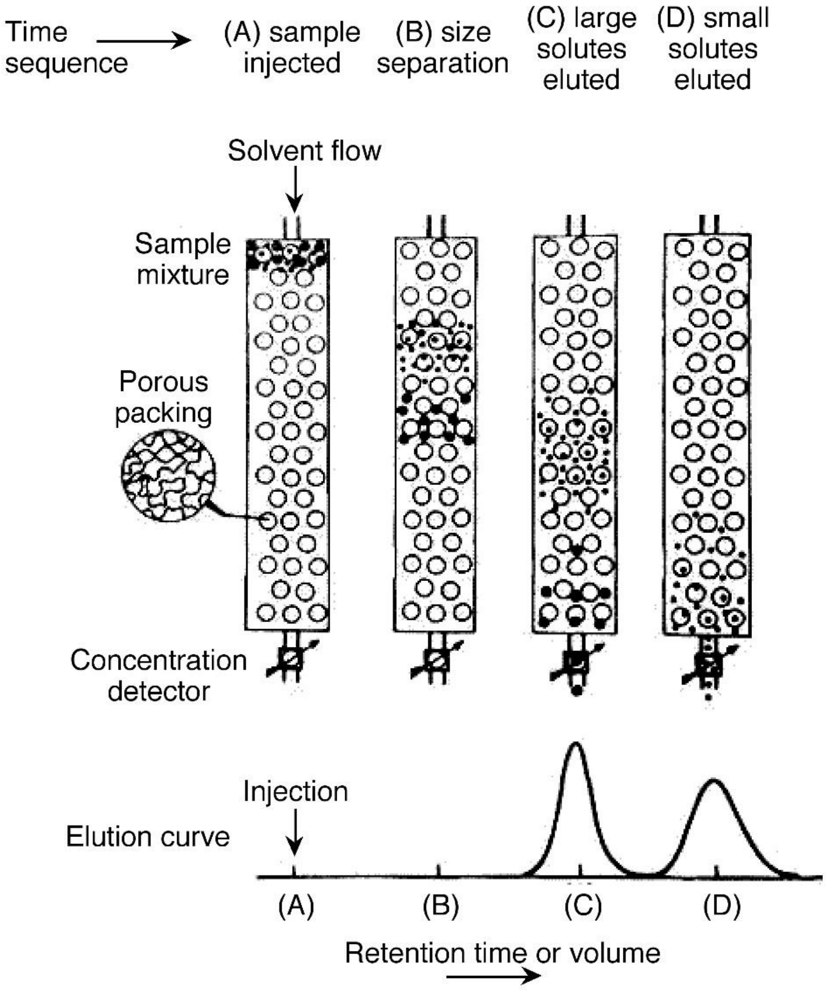
Gels are used as stationary phase for GPC. The pore size of a gel must be carefully controlled in order to be able to apply the gel to a given separation. Other desirable properties of the gel forming agent are the absence of ionizing groups and, in a given solvent, low affinity for the substances to be separated. Commercial gels like PLgel, Sephadex, Bio-Gel (cross-linked polyacrylamide), agarose gel and Styragel are often used based on different separation requirements.

Column

The column used for GPC is filled with a microporous packing material. The column is filled with the gel.

Eluent

The eluent (mobile phase) should be a good solvent for the polymer, should permit high detector response from the polymer and should wet the packing surface. The most common eluents in for polymers that dissolve at room temperature GPC are tetrahydrofuran (THF), dichlorobenzene and trichlorobenzene at 130–150 °C for crystalline polyalkynes and m-cresol and o-chlorophenol at 90 °C for crystalline condensation polymers such as polyamides and polyesters.



Solvent flow through column. Adapted from A. M. Striegel, et.al. Modern Size-Exclusion Liquid Chromatography- Practice of Gel Permeation and Gel Filtration Chromatography

Pump

There are two types of pumps available for uniform delivery of relatively small liquid volumes for GPC: piston or peristaltic pumps.

Detector

In GPC, the concentration by weight of polymer in the eluting solvent may be monitored continuously with a detector. There are many detector types available and they can be divided into two main categories. The first is concentration sensitive detectors which includes UV absorption, differential refractometer (DRI) or refractive index (RI) detectors, infrared (IR) absorption and density detectors. The second category is molecular weight sensitive detectors, which include low angle light scattering detectors and multi angle light scattering. The resulting chromatogram is therefore a weight distribution of the polymer as a function of retention volume. The most sensitive detector is the differential UV photometer and the most common detector is the differential refractometer (DRI). When characterizing copolymer, it is necessary to have two detectors in series. For accurate determinations of copolymer composition at least two of those detectors should be concentration detectors. The determination of most copolymer compositions is done using UV and RI detectors, although other combinations can be used.

Data analysis

GPC has become the most widely used technique for analyzing polymer samples in order to determine their molecular weights and weight distributions. Examples of GPC chromatograms of polystyrene samples with their molecular weights and dispersity are shown on the left. By determining the retention volumes (or times) of monodisperse polymer standards (e.g. solutions of monodispersed polystyrene in THF), a calibration curve can be obtained by plotting the logarithm of the molecular weight versus the retention time or volume. Once the calibration curve is obtained, the gel permeation chromatogram of any other polymer can be obtained in the same solvent and the molecular weights (usually M_n and M_w) and the complete molecular weight distribution for the polymer can be determined. A typical calibration curve is shown to the right and the molecular weight from an unknown sample can be obtained from the calibration curve.

Application of gel permeation chromatography

GPC is often used to determine the relative molecular weight of polymer samples as well as the distribution of molecular weights. GPC truly measures the molecular volume and shape function that is defined by the intrinsic viscosity. If comparable standards are used, this relative data can be used to determine molecular weights within ± 5% accuracy.

Polystyrene standards with dispersity of less than 1.2 are typically used to calibrate the GPC. Unfortunately, polystyrene tends to be a very linear polymer and therefore as a standard it is only useful to compare it to other polymers that are known to be linear and of relatively the same size.

Check Your Progress – 4

1. What is ion exchange chromatography?
2. Write the application of ion exchange chromatography.
3. Explain gel permeation chromatography.
4. What is the role of Eluent in GPC?

12.5 ANSWER TO CHECK YOUR PROGRESS QUESTION

1. Ion-exchange chromatography (IEC) is part of ion chromatography which is an important analytical technique for the separation and determination of ionic compounds, together with ion-partition/interaction and ion-exclusion chromatography
2. Ion exchange chromatography can be used to separate proteins because they contain charged functional groups. The main applications are reliability, very good accuracy and precision, high selectivity, high speed, high separation efficiency, and low cost of consumables.

3. Gel permeation chromatography (GPC) is a type of size exclusion chromatography (SEC), that separates analytes on the basis of size. The technique is often used for the analysis of polymers.
4. The eluent (mobile phase) should be a good solvent for the polymer, should permit high detector response from the polymer and should wet the packing surface. The most common eluents for polymers that dissolve at room temperature are tetrahydrofuran (THF), dichlorobenzene.

12.6 SUMMARY

Ion Chromatography is a method of separating ions based on their distinct retention rates in a given solid phase packing material. It has two different types of chromatography such as ion exchange chromatography and gel permeation chromatography. Ion exchange chromatography is a separation of sample components after their distribution between two phases. It is a type of adsorption chromatography. It is the effective method for the water purification. GC is one of the separation techniques based on the difference in molecular size or weight and it is one of the effective methods to analyse the bio and macromolecular substances. It has many advantages such as narrow band gaps, well defined separation, short analysis time, the flow rate can be set and no sample will loss.

12.7 KEY WORDS

- ❖ Ion exchanger
- ❖ Resins
- ❖ Elution

12.8 SELF-ASSESSMENT QUESTION AND EXERCISES

1. Briefly explain the principle, theory and instrumentation of Ion exchange chromatography?
2. what are the advantages of Ion exchange chromatography?
3. Draw and explain the gel permeation chromatography system.
4. Write some applications for GPC.

12.9 FURTHER READINGS

1. King, C.J. Separation Processes, Tata McGraw - Hill Publishing Co., Ltd., 1982.
2. Ronald W.Roussel - Handbook of Separation Process Technology, John Wiley, New York, 1987.

Purification
Techniques

NOTES

UNIT 13 - PURIFICATION TECHNIQUES

Structure

13.1 INTRODUCTION

Purity is a matter of degree. Other than contaminants such as dust, paper fibres, wax, cork, etc., that may have been inadvertently introduced into the sample during manufacture, all commercially available chemical substances are in some measure impure. Any amounts of unreacted starting material, intermediates, by-products, isomers and related compounds may be present depending on the synthetic or isolation procedures used for preparing the substances. Purification in a chemical context is the physical separation of a chemical substance of interest from foreign or contaminating substances. Since ancient times, people have used methods of isolating and purifying chemical substances for improving the quality of life. The extraction of metals from ores and of medicines from plants is older than recorded history. In the Middle Ages the alchemists' search for the philosophers' stone (a means of changing base metals into gold) and the elixir of life (a substance that would perpetuate youth) depended on separations. In the industrial and technological revolutions, separations and purifications have assumed major importance.

13.2 OBJECTIVES

After going through this unit, you will be able to

- ❖ Understand the concept of isolation and purification of chemicals
- ❖ Know the available methods for the purifications of chemical substance
- ❖ Know in detail about isolations based on phase equilibria
- ❖ Gain the knowledge on purification of solvents

13.3 GENERAL METHODS OF ISOLATION AND PURIFICATION OF CHEMICALS

The purification of organic compounds is necessary, though complex, step after its extraction from a natural source or synthesis in the laboratory. The method of purification of the organic compound depends mainly on the nature of the compound and the impurities present. Solvents and substances that are specified as pure for a particular purpose may, in fact, be quite impure for other uses. Irrespective of the grade of material to be purified, it is essential that some criteria exist for assessing the degree of purity of the final product. The more common of these include:

1. Examination of physical properties such as:
    (a) Melting point, freezing point, boiling point, and the freezing curve (i.e. the variation, with time, in the freezing point of a substance that is being slowly and continuously frozen).
    (b) Density.
    (c) Refractive index at a specified temperature and wavelength.
    (d) Specific conductivity. (This can be used to detect, for example, water, salts, inorganic and organic acids and bases, in non-electrolytes).
    (e) Optical rotation, optical rotatory dispersion and circular dichroism.
2. Empirical analysis, for C, H, N, ash, etc.
3. Chemical tests for particular types of impurities, e.g. for peroxides in aliphatic ethers (with acidified KI), or for water in solvents (quantitatively by the Karl Fischer method)
4. Physical tests for particular types of impurities:
    (a) Emission and atomic absorption spectroscopy for detecting organic impurities and determining metal ions.
    (b) Chromatography, including paper, thin layer, liquid (high, medium and normal pressure), flash and vapour phase.
    (c) Electron spin resonance for detecting free radicals.
5. Examination of spectroscopic properties
6. Electrochemical methods
7. Nuclear methods which include a variety of radioactive elements as in organic reagents, complexes or salts.

The following is the list of purification methods available for chemical compounds;

- ❖ Affinity purification purifies proteins by retaining them on a column through their affinity to antibodies, enzymes, or receptors that have been immobilised on the column.
- ❖ Filtration is a mechanical method to separate solids from liquids or gases by passing the feed stream through a porous sheet such as a cloth or membrane, which retains the solids and allows the liquid to pass through.
- ❖ Centrifugation is a process that uses an electric motor to spin a vessel of fluid at high speed to make heavier components settle to the bottom of the vessel.

- ❖ Evaporation removes volatile liquids from non-volatile solutes, which cannot be done through filtration due to the small size of the substances.

- Liquid–liquid extraction removes an impurity or recovers a desired product by dissolving the crude material in a solvent in which other components of the feed material are soluble.
- Crystallization separates a product from a liquid feed stream, often in extremely pure form, by cooling the feed stream or adding precipitants that lower the solubility of the desired product so that it forms crystals. The pure solid crystals are then separated from the remaining liquor by filtration or centrifugation.
- Recrystallization: In analytical and synthetic chemistry work, purchased reagents of doubtful purity may be recrystallized, e.g. dissolved in a very pure solvent, and then crystallized, and the crystals recovered, in order to improve and/or verify their purity.
- Trituration removes highly soluble impurities from usually solid insoluble material by rinsing it with an appropriate solvent.
- Adsorption removes a soluble impurity from a feed stream by trapping it on the surface of a solid material, such as activated carbon, that forms strong non-covalent chemical bonds with the impurity.
- Chromatography employs continuous adsorption and desorption on a packed bed of a solid to purify multiple components of a single feed stream. In a laboratory setting, mixture of dissolved materials are typically fed using a solvent into a column packed with an appropriate adsorbent, and due to different affinities for solvent (moving phase) versus adsorbent (stationary phase) the components in the original mixture exit the column in the moving phase at different rates, which thus allows to selectively collect desired materials out of the initial mixture.
- Smelting produces metals from raw ore, and involves adding chemicals to the ore and heating it up to the melting point of the metal.
- Refining is used primarily in the petroleum industry, whereby crude oil is heated and separated into stages according to the condensation points of the various elements.
- Distillation, widely used in petroleum refining and in purification of ethanol separates volatile liquids on the basis of their relative volatilities. It is of different types Simple distillation, steam distillation etc.
- Water purification combines a number of methods to produce potable or drinking water.
- Downstream processing refers to purification of chemicals, pharmaceuticals and food ingredients produced by fermentation or synthesized by plant and animal tissues, for example antibiotics, citric acid, vitamin E, and insulin.
- Fractionation refers to a purification strategy in which some relatively inefficient purification method is repeatedly applied to isolate the desired substance in progressively greater purity.
- Electrolysis refers to the breakdown of substances using an electric current. This removes impurities in a substance that an electric current is run through

Purification Techniques

NOTES

- Sublimation is the process of changing of any substance (usually on heating) from a solid to a gas (or from gas to a solid) without passing through liquid phase. In terms of purification - material is heated, often under vacuum, and the vapors of the material are then condensed back to a solid on a cooler surface. The process thus in its essence is similar to distillation, however the material which is condensed on the cooler surface then has to be removed mechanically, thus requiring different laboratory equipment.
- Bioleaching is the extraction of metals from their ores through the use of living organisms.

Classification of various methods

There are a variety of criteria by which separations can be classified. One is based on the quantity of material to be processed. Some methods of separation (e.g., chromatography) work best with a small amount of sample, while others (e.g., distillation) are more suited to large-scale operations.

Table 13.3.1 Isolation and purification based on rate phenomena

Barrier isolations	**Field isolations**
Membrane filtration	Electrophoresis
Dialysis	Ultracentrifugation
Ultrafiltration	Electrolysis
Electrodialysis	Field-flow fractionation
Reverse osmosis	

Classification may also be based on the physical or chemical phenomena utilized to effect the separation. These phenomena can be divided into two broad categories, one is based on rate phenomena (Table 13.3.1) and other is based on phase equilibria (Table 13.3.2)

Table 13.3.2 Isolation and purification based on phase equilibria

Gas-liquid	**Gas-solid**	**Liquid-solid**	**Liquid-liquid**	**Supercritical fluid-solid**	**Supercritical fluid-liquid**
Distillation Gas-liquid chromatography Foam fractionation	Adsorption Sublimation	Precipitation Zone melting Crystallization Ion exchange Adsorption Exclusion Clathration	Extraction Partition chromatography	Supercritical-fluid chromatography	supercritical-fluid extraction

It is important to note that more than one method of purification may need to be implemented in order to obtain compounds of highest purity. A number of these methods are routinely used in synthetic as well as analytical chemistry and biochemistry. Few techniques with greater detail in the respective sections in this chapter are outlined below:

NOTES

Purification
Techniques

NOTES

- Solvent extraction
- Fractional crystallization
- Sublimation
- Distillation
- Vacuum distillation
- Purification of solvents

13.4 SOLVENT EXTRACTION

Solvent extraction is a technique extensively utilized in both industrial applications and in the laboratory. It includes a variety of techniques such as liquid liquid extraction (LLE), liquid solid extraction (LSE), supercritical fluid extraction (SFE), and other special techniques. LLE is an extraction technique applied to liquids, liquid samples, or samples in solution, using a liquid extracting medium.

Principle

The basis of extractive techniques is the "like dissolves like" rule. Water typically dissolves inorganic salts (such as lithium chloride) and other ionized species, while solvents (ethyl acetate, methylene chloride, diethyl ether, etc.) dissolve neutral organic molecules. However, some compounds (e.g., alcohols) exhibit solubility in both media. Therefore, it is important to remember that this method of separation relies on partitioning, that is, the preferential dissolution of a species into one solvent over another. For example, 2-pentanol is partially soluble in water (i.e., 17 g/100mLH_2O), but infinitely soluble in diethyl ether. Thus 2-pentanol can be preferentially partitioned into ether.

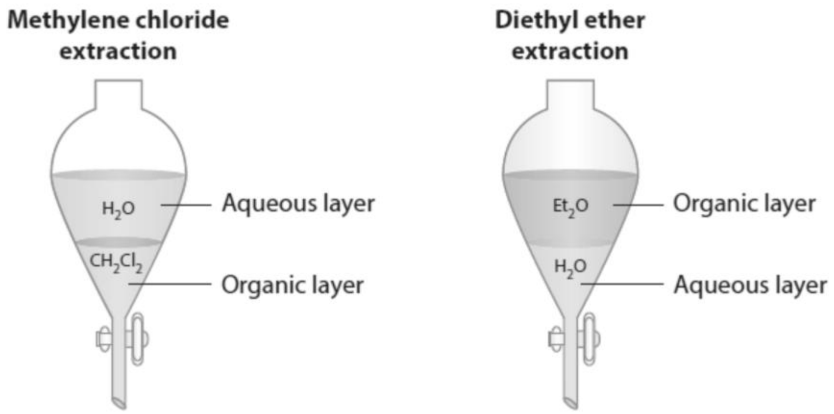


Figure 13.4.1 Organic and aqueous extraction

Extraction process

One of the most common uses of extraction is during aqueous workup, as a way to remove inorganic materials from the desired organic product. On a practical note, workup is usually carried out in a biphasic system i.e. using two immiscible solvents. If a reaction has been carried out in tetrahydrofuran, dioxane, or methanol, then it is generally desirable to remove those solvents by evaporation before workup because they have high solubilities in both aqueous and organic phases, and can set up single phase systems (i.e., nothing to separate) or emulsions.

Typical extraction solvents include ethyl acetate, hexane, chloroform, methylene chloride, and diethyl ether. All of these form crisp delineations between phases. The two layers are commonly referred to as the aqueous phase and the organic phase. It is important to keep track of the phases, as their positions are solvent dependent. For example, diethyl ether is lighter than water, so the organic phase will rest on top in the separatory funnel, whereas methylene chloride is heavier than water and so will sink to the bottom (Figure 13.4.1). the combination of immiscible solvents suitable for extractions are given in Table 13.4.1.

By way of vocabulary, actually two operations are encountered in the separatory funnel. When components are removed from an organic layer by shaking with an aqueous solution, the organic phase is said to be washed (e.g., "The combined ether extracts were washed with aqueous sodium bicarbonate solution"). On the other hand, when components are removed from water by treatment with an organic solvent, the aqueous phase is said to be extracted (e.g., "The aqueous layer was extracted with three portions of ethyl acetate"). Thus aqueous layers are extracted, and organic layers are washed—although these two terms are sometimes (erroneously) used interchangeably.

Addition of electrolytes (such as ammonium sulfate, calcium chloride or sodium chloride) to the aqueous phase helps to ensure that the organic layer separates cleanly and also decreases the extent of extraction into the latter. Emulsions can also be broken up by filtration (with suction) through Celite, or by adding a little diethyl ether, octyl alcohol or some other paraffinic alcohol. The main factor in selecting a suitable immiscible solvent is to find one in which the material to be extracted is readily soluble, whereas the substance from which it is being extracted is not. The same considerations apply irrespective of whether it is the substance being purified, or one of its contaminants, that is taken into the new phase.

Table 13.4.1 Some common immiscible or slightly miscible Pairs of solvents

Carbon tetrachloride with ethanolamine, ethylene glycol, formamide or water.
Dimethyl formamide with cyclohexane or petroleum ether.
Dimethyl sulfoxide with cyclohexane or petroleum ether.
Ethyl ether with ethanolamine, ethylene glycol or water.
Methanol with carbon disulfide, cyclohexane or petroleum ether.
Petroleum ether with aniline, benzyl alcohol, dimethyl formamide, dimethyl sulfoxide, formamide, furfuryl alcohol, phenol or water.
Water with aniline, benzene, benzyl alcohol, carbon disulfide, carbon tetrachloride, chloroform, cyclohexane, cyclohexanol, cyclohexanone, diethyl ether, ethyl acetate, isoamyl alcohol, methyl ethyl ketone, nitromethane, tributyl phosphate or toluene.

Washing

Washing process can also be applied to purification of the substance if it is an acid, a phenol or a base, by extracting into the appropriate aqueous solution to form the salt which, after washing with pure solvent, is again converted to the free species and re-extracted.

Paraffin hydrocarbons can be purified by extracting them with phenol (in which aromatic hydrocarbons are highly soluble) prior to fractional distillation. For extraction of solid materials with a solvent, a Soxhlet extractor is commonly used. This technique is applied, for example, in the alcohol extraction of dyes to free them from insoluble contaminants such as sodium chloride or sodium sulfate.

Organic solvents are used for workup because they are easily removed by evaporation, leaving behind the organic compound of interest. A common problem encountered at this point is residual water from the aqueous washes. Ethyl acetate and diethyl ether both dissolve large quantities of water (3.3% and 1.2%, respectively). Therefore, it is advantageous to wash these organic layers with brine (saturated NaCl solution) at the end of the extraction sequence, the brine draws out the dissolved water through an osmotic-like effect. For methylene chloride and chloroform, a brine wash is unnecessary, since the solubility of water in these solvents is quite low. Once freed from the bulk of residual water, the organic layer is dried over a desiccant, such as sodium sulfate, calcium chloride, or magnesium sulfate, and then decanted or filtered before evaporation.

13.5 FRACTIONAL CRYSTALLIZATION

The fractional crystallization is a method of refining substances based on differences in their solubility. It fractionates via differences in crystallization (forming of crystals). If a mixture of two or more substances in solution are allowed to crystallize, for example by allowing the temperature of the solution to decrease or increase, the precipitate will contain more of the least soluble substance. The proportion of components in the precipitate will depend on their solubility products. If the solubility products are very similar, a cascade process will be needed to effectuate a complete separation. This technique is often used in chemical engineering to obtain very pure substances, or to recover saleable products from waste solutions. A single crystallisation operation performed on a solution or a melt may fail to produce a pure crystalline product for a variety of reasons including:

- the impurity may have solubility characteristics similar to those of the desired pure component, and both substances consequently co-crystallise,
- the impurity may be present in such large amounts that the crystals inevitably become contaminated.
- a pure substance cannot be produced in a single crystallisation stage if the impurity and the required substance form a solid solution.
- Re-crystallisation from a solution or a melt is, therefore, widely employed to increase crystal purity

13.5.1 Principle

Fractional crystallization is a stagewise separation technique that relies upon liquid-solid phase transition and enables multicomponent mixtures to be split into narrow fractions, ultimately leading to top purities of selected components, through the virtue of selectivity found in solid liquid equilibria. Fractional crystallization is most frequently encountered

in the separation of organic materials ranging from isomer separations to tar chemical mixtures and from organic acids to monomers.

13.5.2 Liquid Phase Structure

Crystallization can be performed both from melts and from solutions. The addition of solvents generally reduces the operating temperature and increases the selectivity of purification. The presence of solvents leads to reduced viscosity and, in some cases, modifies crystal habit. On the other hand, the absence of a solvent involves smaller equipment volumes and does not require additional equipment to recover the solvent.

13.5.3 Phase Equilibria

Solid liquid phase equilibria are shown in phase diagrams and classified as eutectic or solid solutions. In multicomponent systems many combinations of eutectic and solid solution behaviour are known. In the absence of published equilibrium data, extensive laboratory and pilot testing are prerequisites for successful process design. The majority of binary and multicomponent systems form eutectics which theoretically allow crystallization of a specific pure component in a single step. Even where solid solutions occur, separation factors are often higher than those attainable by distillation. Melt crystallization does not require a solvent. The feedstock is heated until molten, and the melt is then cooled until partially frozen.

13.5.4 Crystallization techniques

Industrial crystallization features generation of crystals by nucleation, crystal growth and the recovery of crystals from residual mother liquor. Both generation and recovery of crystals can be performed either from suspension or through a crystal layer.

Crystallization in Suspension

With this technique a melt or a solution is cooled below saturation temperature. Crystals grow under adiabatic conditions with supersaturation as driving force being present throughout the liquid phase. A very special knowhow is required to secure crystal shape, crystal size and crystal size distribution. Since the product from suspension crystallization is a slurry, the separation of residual mother liquor from large specific surface area is usually carried out in dedicated equipment.

Layer Crystallization

Crystals are allowed to grow onto a cooled surface. Crystals grow in a non-adiabatic environment in such a way that subcooling is supplied through the crystal layer rather than through the liquid phase. The driving force results from the net effect of temperature and concentration gradients across both solid and liquid phase. Under these conditions crystal growth rate is 10 to 100 times faster than in suspension crystallization. Generally two different systems used are Static Crystallization: crystals are grown from a stagnant melt and Falling Film Crystallization: the liquid is moved in relation to the solid surface.

Application

- ❖ Fractional crystallization is also important in the formation of sedimentary evaporite rocks.
- ❖ Fractional crystallization is used for refining of molten aluminium scrap.
- ❖ Fractional crystallization can be used to separate solid-solid mixtures. An example is separating KNO3 and KClO3.

13.6 SUBLIMATION

Some compounds are capable of sublimation, which is the direct phase change from solid to gas. Solid carbon dioxide is an example of a substance that sublimes readily at atmospheric pressure, as a chunk of dry ice will not melt, but will seem to "disappear" as it turns directly into carbon dioxide gas. Examples of compounds that can be sublimed are camphor, benzophenone, naphthalene, anthracene, pyrene, etc.

Principle

Sublimation is an analogous process to boiling, as it occurs when a compound's vapor pressure equals its applied pressure (often the atmospheric pressure). The difference is that sublimation involves a solid's vapor pressure instead of a liquid's.

Most solids do not have an appreciable vapor pressure at easily accessible temperatures, and for this reason the ability to sublime is uncommon. Compounds that are capable of sublimation tend to be those with weak intermolecular forces in the solid state. Figure 13.6.1 shows the general experimental set up for sublimation.

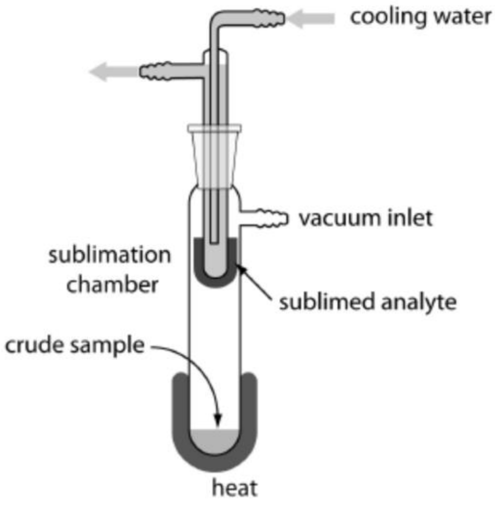


Figure 13.6.1 Experimental setup for a sublimation

Sublimation process

As relatively few solids are capable of sublimation, the process can be an excellent purification method when a volatile solid is contaminated with non-volatile impurities. The impure solid is heated in the bottom of a vessel in close proximity to a cold surface, called a "cold finger" (Figure 13.6.2).

NOTES

As the volatile solid sublimes, it is deposited on the surface of the cold finger (where it can later be recovered), and is thus separated from the non-volatile substance left in the vessel. Sublimation is an example of a "green chemistry" technique, as no solvents are used and no waste is generated. The process, however, is not particularly efficient at separating volatile solids from one another.

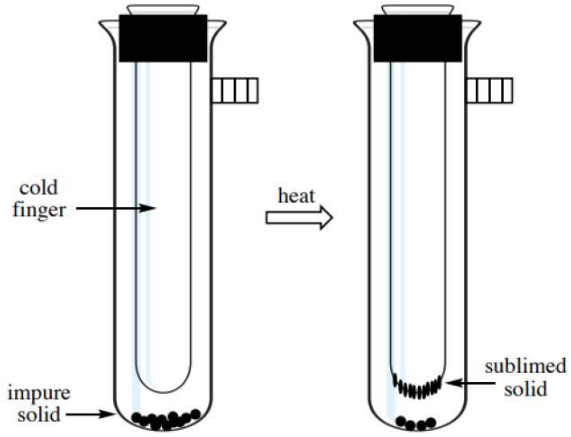


Figure 13.6.2 Diagram for the sublimation process

Of the solids with appreciable vapor pressures at room temperature, many still require rather high temperatures to actively sublime (when their vapor pressure equals the atmospheric pressure of nearly 760 mm Hg). If these solids are heated to their sublimation points under atmospheric pressure, some will char and decompose during the process. For this reason, it is very common to perform sublimation under a reduced pressure (vacuum sublimation). Analogous to vacuum distillation in which liquid boils when its vapor pressure equals the reduced pressure in the apparatus, in vacuum sublimation solid sublimes when its vapor pressure equals the reduced pressure in the apparatus. In vacuum distillation, reducing the pressure allows for liquids to boil at a lower temperature. Similarly, reducing the pressure in vacuum sublimation allows for solids to sublime at a lower temperature, one which avoids decomposition.

Applications

- Drying: Sublimation helps to dry substance within getting moist. This process of sublimation is also beneficial in medicine. It is used in making powders from heat sensitive materials etc.
- Purification: If is a substance is in contaminated form, it can be separated into pure form by sublimation technique. since the sublimation temperature of substance is different than the contaminant, when sublimation is done, the substance is separated leaving the contaminant behind.
- Aroma: Sublimation is the principle involved in air freshener solids used in room and toilets. The room fresheners are solids packed in a box. They spread the odor to the entire room by getting evaporated.

13.7 DISTILLATION

One of the most widely applicable and most commonly used methods of purification of liquids or low melting solids (especially of organic chemicals) is distillation at atmospheric, or some lower, pressure. Almost without exception, this method can be assumed to be suitable for all organic liquids and most of the low-melting organic solids. Figure 13.7.1 shows the general experimental set up for distillation.

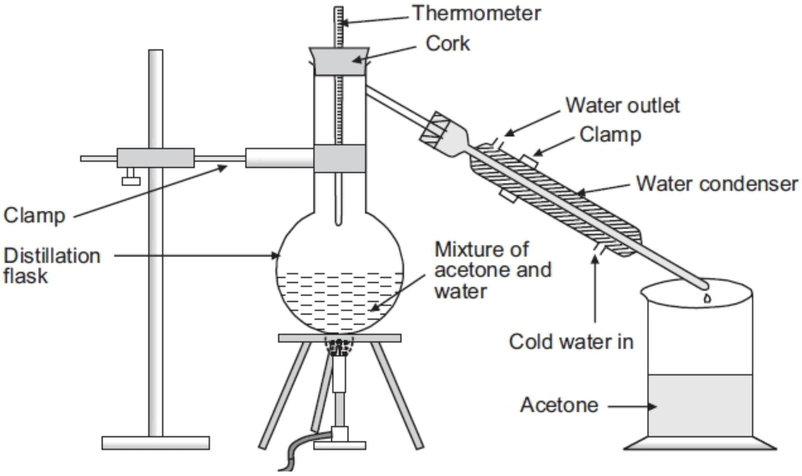


Figure 13.7.1 Experimental setup for distillation process

Principle

Distillation is a purification method for liquids, and can separate components of a mixture if they have significantly different boiling points. In a distillation, a liquid is boiled in the "distilling flask," then the vapors travel to another section of the apparatus where they come into contact with a cool surface. The vapors condense on this cool surface, and the condensed liquid (called the "distillate") drips into a reservoir separated from the original liquid. In the simplest terms, a distillation involves boiling a liquid, then condensing the gas and collecting the liquid elsewhere.

The boiling point of a liquid varies with the 'atmospheric' pressure to which it is exposed. A liquid boils when its vapour pressure is the same as the external pressure on its surface, its normal boiling point being the temperature at which its vapour pressure is equal to that of a standard atmosphere (760mm Hg). Lowering the external pressure lowers the boiling point. For most substances, boiling point and vapour pressure are related by an equation of the form,

$$\log p = A + B/(t + 273)$$

where p is the pressure in mmHg, t is in oC, and A and B are constants.

Hence, if the boiling points at two different pressures are known, the boiling point at another pressure can be calculated from a simple plot of log p versus 1/(t + 273). For organic molecules that are not strongly associated, this equation can be written in the form,

$$\log p = 8.586 - 5.703 (T + 273)/(t + 273)$$

where T is the boiling point in oC at 760mm Hg.

Distillation technique

The distillation apparatus consists basically of a distillation flask, usually fitted with a vertical fractionating column (which may be empty or packed with suitable materials such as glass helices or stainless-steel wool) to which is attached a condenser leading to a receiving flask. The bulb of a thermometer projects into the vapour phase just below the region where the condenser joins the column. The distilling flask is heated so that its contents are steadily vaporised by boiling. The vapour passes up into the column where, initially, it condenses and runs back into the flask. The resulting heat transfer gradually warms the column so that there is a progressive movement of the vapour phase-liquid boundary up the column, with increasing enrichment of the more volatile component. Because of this fractionation, the vapour finally passing into the condenser (where it condenses and flows into the receiver) is commonly that of the lowest-boiling components in the system. The conditions apply until all of the low-boiling material has been distilled, whereupon distillation ceases until the column temperature is high enough to permit the next component to distil. This usually results in a temporary fall in the temperature indicated by the thermometer.

Distilling flask

To minimise superheating of the liquid (due to the absence of minute air bubbles or other suitable nuclei for forming bubbles of vapour), and to prevent bumping, one or more of the following precautions should be taken:

- The flask is heated uniformly over a large part of its surface, either by using an electrical heating mantle or, by partial immersion in a bath above the boiling point of the liquid to be distilled.
- Before heating begins, small pieces of unglazed fireclay or porcelain (porous pot, boiling chips), pumice, diatomaceous earth, or platinum wire are added to the flask. These act as sources of air bubbles.
- The flask may contain glass siphons or boiling tubes. The former are inverted J-shaped tubes, the end of the shorter arm being just above the surface of the liquid. The latter comprise long capillary tubes sealed above the lower end.
- A steady slow stream of inert gas (e.g. N2, Ar or He) is passed through the liquid.
- The liquid in the flask is stirred mechanically. This is especially necessary when suspended insoluble material is present.

For simple distillations a Claisen flask is often used. This flask is, essentially, a round-bottomed flask to the neck of which is joined another neck carrying a side arm. This second neck is sometimes extended so as to form a Vigreux column [a glass tube in which have been made a number of

pairs of indentations which almost touch each other and which slope slightly downwards. The pairs of indentations are arranged to form a spiral of glass inside the tube].

Types of columns

Columns used in distillation vary in their shapes and types of packing. Packed columns are intended to give efficient separation by maintaining a large surface of contact between liquid and vapour. Efficiency of separation is further increased by operation under conditions approaching total reflux, i.e. under a high reflux ratio. However, great care must be taken to avoid flooding of the column during distillation. Some of the commonly used columns are;

- Bruun column: A type of all-glass bubble-cap column.
- Bubble-cap column: A type of plate column in which inverted cups (bubble caps) deflect ascending vapour through reflux liquid lying on each plate. Excess liquid from any plate overflows to the plate lying below it and ultimately returns to the flask.
- Dufton column: A plain tube, into which fits closely (preferably ground to fit) a solid glass spiral wound round a central rod.
- Hempel column: A plain tube (fitted near the top with a side arm) which is almost filled with a suitable packing, which may be of rings or helices.
- Oldershaw column: An all-glass perforated-plate column. The plates are sealed into a tube, each plate being equipped with a baffle to direct the flow of reflux liquid, and a raised outlet which maintains a definite liquid level on the plate and also serves as a drain on to the next lower plate.
- Podbielniak column: A plain tube containing "Heli-Grid" Nichrome or Inconel wire packing. This packing provides a number of passage-ways for the reflux liquid, while the capillary spaces ensure very even spreading of the liquid, so that there is a very large area of contact between liquid and vapour while, at the same time, channelling and flooding are minimized.
- Stedman column: A plain tube containing a series of wire-gauze discs stamped into flat, truncated cones and welded together, alternatively base-to-base and edge-to-edge, with a flat disc across each base.
- Todd column: A column which is surrounded by an open heating jacket so that the temperature can be adjusted to be close to the distillation temperature.
- Vigreux column: A glass tube in which have been made a number of pairs of indentations which almost touch each other and which slope slightly downwards. The pairs of indentations are arranged to form a spiral of glass inside the tube
- Widmer column: A Dufton column, modified by enclosing within two concentric tubes the portion containing the glass spiral. Vapour passes up the outer tube and down the inner tube before entering the centre portion.

Types of condensers

- Air condenser: A glass tube such as the inner part of a Liebig condenser. Used for liquids with boiling points above 90o. Can be of any length.

- Allihn condenser: The inner tube of a Liebig condenser is modified by having a series of bulbs to increase the condensing surface. Further modifications of the bubble shapes give the Julian and Allihn-Kronbitter condensers.
- Bailey-Walker condenser: A type of all-metal condenser fitting into the neck of extraction apparatus and being supported by the rim. Used for high-boiling liquids.
- Coil condenser: An open tube, into which is sealed a glass coil or spiral through which water circulates. The tube is sometimes also surrounded by an outer cooling jacket.
- Double surface condenser: A tube in which the vapour is condensed between an outer and inner water cooled jacket after impinging on the latter.
- Friedrichs condenser: A "cold-finger" type of condenser sealed into a glass jacket open at the bottom and near the top. The cold finger is formed into glass screw threads.
- Graham condenser: A type of coil condenser.
- Hopkins condenser: A cold-finger type of condenser resembling that of Friedrichs.
- Liebig condenser: An inner glass tube surrounded by a glass jacket through which water is circulated.
- Other condenser: A large-capacity condenser which has two coils of relatively large bore glass tubing inside it, through which the water flows. The two coils join at their top and bottom.
- West condenser: A Liebig condenser with a light-walled inner tube and a heavy-walled outer tube, with only a narrow space between them.
- Wiley condenser: A condenser resembling the Bailey-Walker type.

Application

Simple distillation is the primary method used for purifying drinking water on both a large and small scale. There are many models available for purifying water on a smaller scale. Simple distillation is also a primary method for the purification of ethanol into fuel grade alcohol. The alcohol industry uses simple distillation to improve the concentration of alcohol.

13.8 VACUUM DISTILLATION

Boiling commences when the vapor pressure of a liquid or solution equals the external or applied pressure (often atmospheric pressure). Thus, if the applied pressure is reduced, the boiling point of the liquid decreases. This behavior occurs because a lower vapor pressure is necessary for boiling, which can be achieved at a lower temperature. The dependence of boiling point on applied pressure can be exploited in the distillation of very high boiling compounds (normal b.p. > 150 ^{o}C), which may decompose if heated to their normal boiling point. A vacuum distillation is performed by applying a vacuum source to the vacuum adapter of either a simple or fractional distillation (Figure 13.8.1). When the pressure is lowered inside the apparatus, solutions boil at a lower temperature. Generally this method is employed when the difference between the boiling points of the liquids isn’t much. Since the vapours of such liquids might condense together, a fractionating column is fixed to the mouth of the RB.

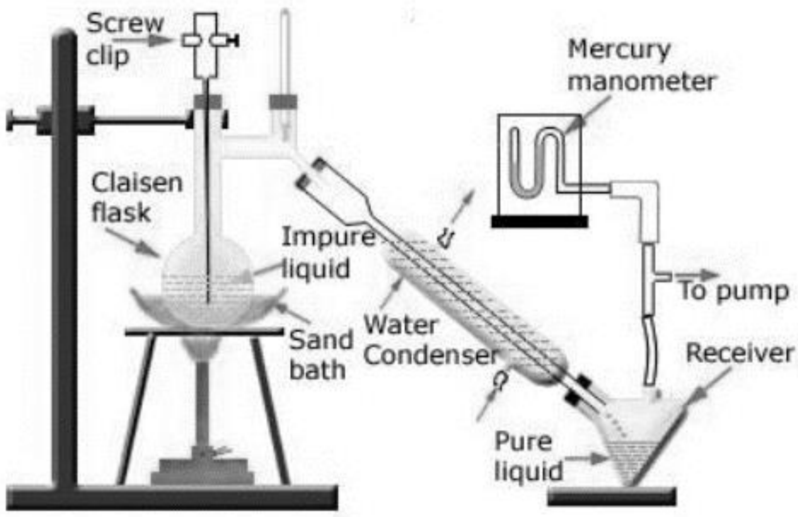


Figure 13.8.1 Experimental setup for vacuum distillation process

Principle

Vacuum distillation is distillation performed under reduced pressure, which allows the purification of compounds not readily distilled at ambient pressures or simply to save time or energy. This technique separates compounds based on differences in boiling points. This technique is used when the boiling point of the desired compound is difficult to achieve, will cause the compound to decompose. A reduced pressure decreases the boiling point of compounds. The reduction in boiling point can be calculated using a temperature-pressure nomograph using the Clausius–Clapeyron relation.

Predicting the boiling temperature

The boiling point of a liquid or solution drops when the pressure is reduced in a distillation apparatus. It is helpful to be able to predict the altered boiling point depending on the pressure inside the apparatus. The lowest pressure attainable inside the apparatus depends largely on the vacuum source and the integrity of the seal on the joints. Lower pressures are attainable when using a portable vacuum pump than when using a water aspirator or the building's house vacuum. Due to the very low pressures possible with oil pumps in portable vacuums, these vacuum distillations should be conducted in the fume hood behind a blast shield.

Water aspirators are the most common vacuum source in teaching labs because they are inexpensive. When a water aspirator is used, the vacuum pressure is always limited by the intrinsic vapor pressure of water, which is often between 17.7 mm Hg (20 °C) and 23.8 mm Hg (25 °C). The vacuum pressure is also very dependent on water flow, which can vary greatly. If a manometer is available, the distillation apparatus should be set up and evacuated without heating to measure the pressure. The expected boiling point of a compound can then be roughly estimated using a nomograph. If a manometer is not available and a water aspirator is to be used, the expected boiling point can be estimated using an approximate pressure of 20 mm Hg, although the pressure will likely be higher than this.

NOTES

Procedure for vacuum distillation

- Safety note: Inspect every piece of glassware to be used with the vacuum distillation, checking for stars, cracks, or other weaknesses in the glass, as these may allow for implosion when the pressure is reduced.
- A stir bar needs to be used for bump prevention. Boiling stones cannot be used with vacuum distillation as air trapped in the stone's pores is rapidly removed under vacuum, causing the stones to fail to produce bubbles.
- Although greasing is somewhat of a personal choice with simple and fractional distillations, all joints must be greased in vacuum distillations or the system will leak and fail to achieve a low pressure.
- Begin assembly of the apparatus near the vacuum source. If using a water aspirator, test to be sure that the aspirator works well as some are more functional than others. To test an aspirator, apply thick vacuum hosing to the nub on the aspirator, turn on the water and feel for suction at the end of the hose with finger.
- A Claisen adapter should be included in the apparatus as solutions under vacuum tend to bump violently.
- Attach thick-walled tubing to the vacuum adapter on the distillation apparatus and connect to a vacuum trap. Connect the trap to the vacuum source (aspirator or vacuum pump). It is best to not bend or strain the tubing as much as is practical, as this may create a leak in the system.
- Insert a wood block or lab jack beneath the stirring plate to allow for lowering of the heat source when the distillation is complete.
- Before heating, turn on the vacuum source to begin reducing pressure inside the apparatus.
- When confident that the apparatus is adequately evacuated and any low-boiling compounds have been removed, begin heating the sample.
- If it is difficult to achieve more than a reflux, the Claisen and three-way adapter can be insulated by wrapping them tightly with glass wool then aluminum foil.
- Record the temperature over which material is collected, making sure the value corresponds to a temperature when the thermometer bulb is fully immersed in vapors. If a manometer is used, also record the pressure. If no manometer is used, record the vacuum source (e.g. aspirator).
- If more than one fraction of distillate is desired, the distillation must be stopped before changing the receiving flask.
- To stop the distillation, first remove the heat source, cool the flask to room temperature then further cool in a tap water bath.
- Slowly reinstate the atmospheric pressure into the flask by opening the pinch clamp at the vacuum trap, or by removing the rubber tubing at the vacuum adapter or aspirator.
- Disassemble and clean up the distillation apparatus as quickly as is practical, as the joints can sometimes freeze if left connected for prolonged periods

Applications

- In laboratory, compounds with a boiling point lower than 150 °C can typically be distilled at ambient pressure using rotary evaporation.
- Vacuum distillation is used for petroleum refining, as it is also referred as "low-temperature distillation".
- Molecular distillation is vacuum distillation below the pressure of 0.01 torr (1.3 Pa). 0.01 torr is one order of magnitude above high vacuum, where fluids are in the free molecular flow regime, i.e. the mean free path of molecules is comparable to the size of the equipment.
- Vacuum distillation is often used in large industrial plants as an efficient way to remove salt from ocean water, in order to produce fresh water. This is known as desalination. The ocean water is placed under a vacuum to lower its boiling point and has a heat source applied, allowing the fresh water to boil off and be condensed. The condensing of the water vapor prevents the water vapor from filling the vacuum chamber, and allows the effect to run continuously without a loss of vacuum pressure.

13.9 PURIFICATION OF SOLVENTS

In order to obtain satisfactory, or even any results, in many syntheses it may be necessary to purify solvents to remove reactive impurties such as water or other protic/acidic materials, or atmospheric contaminants such as oxygen or carbon dioxide. Removal of non-gaseous contaminants must be tailored to the specific solvent and often the specific contaminant to be removed. An excellent source for methods of purification of a wide variety of solvents is “Purification of Laboratory Chemicals”. Sometimes simply using a freshly opened bottle of solvent certified by the vendor for a certain level of purity is satisfactory, but when really sensitive reagents are used and/or analytical results are required then organic solvents usually must be purified.

Removal of oxygen and other gaseous atmospheric contaminants (degassing) can be done in several ways. The simplest is to sparge the solvent with a readily available, oxygen free, inert gas such as nitrogen or argon. The length of time required depends upon the amount of solvent to be degassed and may range from a few minutes up to a half h or more. Obviously this requires considerable compressed gas and will result in significant loss of a volatile solvent. It may be inappropriate when only a small amount of solvent is available. It is also not the most effective method.

A better method, and one that would be easily employed when distillation was required for removal of other impurities, is distillation under an inert atmosphere. The process of boiling expels gases from the solvent. If this process is done under an inert atmosphere then the dissolved gases will be expelled and replaced by the inert gas. Note that saturation of an unstirred liquid is quite slow.

If the amount of material available is very small, or if it is thermally sensitive, then degassing by multiple freeze, pump, thaw cycles may be

appropriate. In this process the liquid to be degassed is placed in a vessel equipped with a stopcock or valve that is suitable for evacuation.

The liquid is frozen using an appropriate cold bath and the head space evacuated. The stopcock is closed and the liquid allowed to melt. After a few minutes preferably with swirling of the vessel to achieve equilibration between the liquid and gas phases, it is again frozen and the headspace evacuated. The process is repeated 3-5 times. At this point the liquid should be essentially free of gaseous impurities and can be transferred to the reaction vessel. If this is to be done without exposure to the atmosphere then there must be some provision for breaking the vacuum with an inert gas and bringing the system to atmospheric pressure and provision for removing the liquid with a syringe or other "airless" transfer method.

Some common solvent purification methods

Acetone: Dry over Molecular sieves or K_2CO_3. Removal of aldehydes by $KMnO_4$. High purity: saturate with dry NaI, then chill to -10 °C, filter off NaI crystals. Distillation sieves.

Acetonitrile: Pre-dry if wet, reflux & stir with CaH until gas evolution ceases. Distill and store over sieves.

Benzene: Stir with H_2SO_4 (1l/l benzene) and separate acid. Repeat until no darkening occurs, then distill.

Chloroform: Shake with con H_2SO_4, wash with water, dry and distill from K_2CO_3. Alternatively, pass through column of Grade I activated alumina (50g/L solvent).

Diethyl ether: Check for peroxides. Pretreat with Na wire, then add LAH (or CaH) and distill.

DMF: Stir with KOH, filter and distill from CaO or BaO.

DMSO: Dry overnite with Drierite, BaO, CaO, or NaOH. Distill from BaO, CaO, or NaOH (2-3mm, 50 C).

Ethanol: For anhydrous from abs: Reflux 60 ml EtOH, 5 g Mg, and a few drops of $CHCl_3$ or EtBr (catalyst) until all Mg converted to the oxide. Add 900 ml EtO then distill, store over sieves. Abs EtOH can be prepared from 95% if benzene must be excluded by refluxing over CaO.

Ethyl Acetate: Wash with 5% Aq Na_2CO_3, then saturated $CaCl_2$; dry over K_2CO_3 and distill from P2O5.

Methanol: Most water removed by storage over CaO then distillation from CaO. Storage over sieves (NO DRIERITE!!).

Methylene Chloride: Wash with con. H_2SO_4, saturated Na_2CO_3, and water. Dry over $CaCl_2$ and distill from P_2O_5. Alternatively, distillation from anhydrous K_2CO_3.

Pyridine: Dry over KOH for ~24 h, then distill from BaO or CaO. Store over sieves.

Saturated Hydrocarbons: Most contain small amounts of olefin/aromatic. For removal, shake with a mixture of H_2SO_4 and HNO_3 3-4 times, wash water, dry $CaCl_2$, and distill.

Toluene: Dry over $CaCl_2$ (only if very wet) then distill.

Check Your Progress – 4

1. Name some purification techniques available for chemicals.
2. What is solvent extraction?
3. Explain the principle of fractional crystallization method?
4. Write some application of vacccum distillation

13.10 ANSWER TO CHECK YOUR PROGRESS QUESTION

1. Affinity purification, Filtration, Distillation, Evaporation, Crystallization, Re-Crystallization, Adsorption.
2. Solvent extraction is a method used to separate compounds based on their relative solubilities in two different immiscible liquid usually water and organic solvent.
3. Fractional crystallization is a stagewise separation technique that relies upon liquid-solid phase transition and enables multicomponent mixtures to be split into narrow fractions, most frequently encountered in the separation of organic materials ranging from isomer separations to tar chemical mixtures and from organic acids to monomers.
4. In laboratory, compounds with a boiling point lower than 150 °C can typically be distilled at ambient pressure using rotary evaporation.
5. Vacuum distillation is used for petroleum refining, as it is also referred as "low-temperature distillation"

13.11 SUMMARY

The purification of organic compounds is necessary, though complex, step after its extraction from a natural source or synthesis in the laboratory. The method of purification of the organic compound depends mainly on the nature of the compound and the impurities present. There are a variety of criteria by which separations can be classified. One is based on the quantity of material to be processed. Some methods of separation (e.g., chromatography) work best with a small amount of sample, while others (e.g., distillation) are more suited to large-scale operations. There are many purification techniques such as solvent extraction, fractional crystallization, distillation, sublimation exists. Each exhibit unique properties for the identification, purification and separation process. These techniques are widely used in the industrial areas in every manner.

13.12 KEY WORDS

- ❖ Crystallization
- ❖ Distillation
- ❖ Desalination
- ❖ Solvents

13.13 SELF-ASSESSMENT QUESTION AND EXERCISES

1. State some general methods of isolation and purification of chemicals.
2. Mention some general purification techniques.
3. Explain sublimation process.
4. Discuss about vacuum distillation.
5. Mention some common solvent purification system

13.14 FURTHER READINGS

1. King, C.J. Separation Processes , Tata McGraw - Hill Publishing Co., Ltd., 1982.
2. Ronald W.Roussel - Handbook of Separation Process Technology, John Wiley, New York, 1987.

UNIT 14: IONIC SEPARATIONS

Structure

14.1 INTRODUCTION

The history of separating charged particles and their chemical analysis began with the work of Arne Tiselius in 1931, while new separation processes and chemical analysis techniques based on electrophoresis continue to be developed in the 21st century. Early work with the basic principle of electrophoresis dates to the early 19th century, based on Faraday's laws of electrolysis proposed in the late 18th century and other early electrochemistry. Experiments by Johann Wilhelm Hittorf, Walther Nernst, and Friedrich Kohlrausch to measure the properties and behavior of small ions moving through aqueous solutions under the influence of an electric field led to general mathematical descriptions of the electrochemistry of aqueous solutions. Kohlrausch created equations for varying concentrations of charged particles moving through solution, including sharp moving boundaries of migrating particles. The electrodialysis and electrophoresis are two useful separation methods that allow us to separate charged particles using an electric field in the apparatus.

14.2 OBJECTIVES

After going through this unit, you will be able to

- ❖ know the difference between electrophoresis, di-electrophoresis and electro dialysis
- ❖ know the factors that control the ionic separations
- ❖ know about the applications of different ionic separation techniques.

14.3 ELECTROPHORESIS

Electrophoresis started its history in 1930 when Tiselius published his thesis, “The Moving Boundary Method of Studying the Electrophoresis of Proteins”. In 1948, Tiselius won the Nobel Prize for the development of the moving boundary method and chromatographic adsorption analysis. Electrophoresis in aqueous solutions in the spaces of filter-paper as a supporting medium, paper electrophoresis, became a success from about 1950 thanks to contributions by Wieland, Fischer and others. New electrophoresis methods were also beginning to address some of the

shortcomings of the moving boundary electrophoresis of the Tiselius apparatus.

By the 1960s, increasingly sophisticated gel electrophoresis methods made it possible to separate biological molecules based on minute physical and chemical differences, helping to drive the rise of molecular biology. Since then, electrophoresis methods have diversified considerably, and new methods and applications are still being developed.

Principle

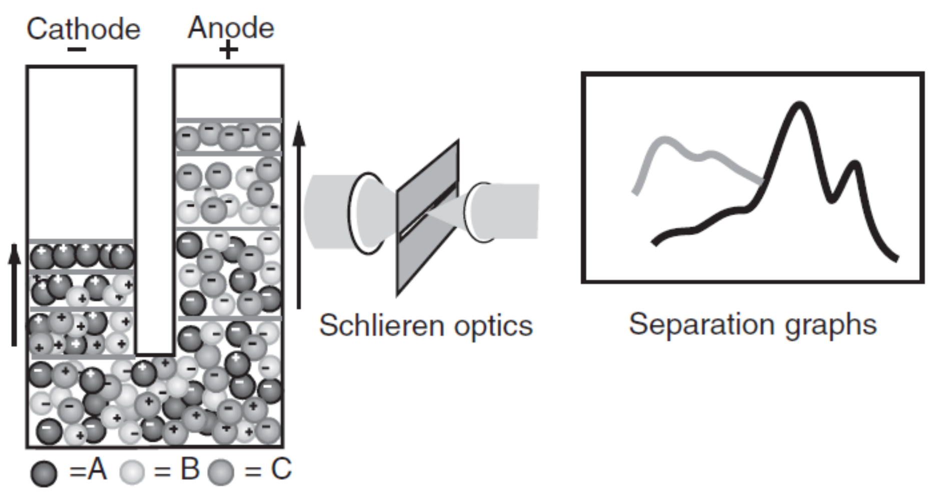
Electrophoresis is a class of separation techniques in which we separate analytes by their ability to move through a conductive medium, usually an aqueous buffer, in response to an applied electric field. In the absence of other effects, cations migrate toward the electric field's negatively charged cathode. Cations with larger charge-to-size ratios which favors ions of larger charge and of smaller size migrate at a faster rate than larger cations with smaller charges. Anions migrate toward the positively charged anode and neutral species do not experience the electrical field and remain stationary.

Types of electrophoresis

There are several forms of electrophoresis. In slab gel electrophoresis the conducting buffer is retained within a porous gel of agarose or polyacrylamide. Slabs are formed by pouring the gel between two glass plates separated by spacers. Typical thicknesses are 0.25–1 mm. Gel electrophoresis is an important technique in biochemistry where it is frequently used for separating DNA fragments and proteins. Although it is a powerful tool for the qualitative analysis of complex mixtures, it is less useful for quantitative work.

Moving boundary electrophoresis:

The sample, a mixture of proteins, is applied into a U-shaped glass tube (Figure 14.3.1) filled with a buffer solution and containing electrodes at each end. Under the influence of the applied electric field, the charged compounds migrate at different velocities toward the anode or the cathode, respectively.



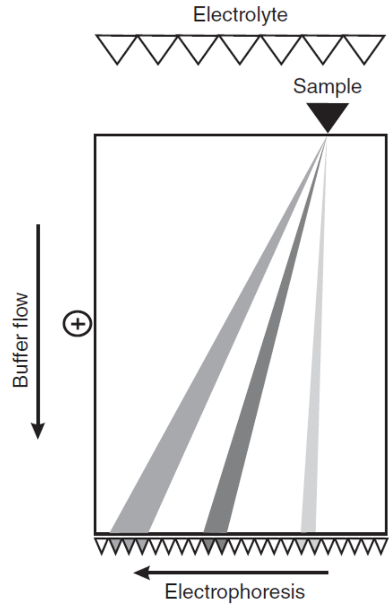
14.3.1 Moving boundary electrophoresis

Ionic
Separations

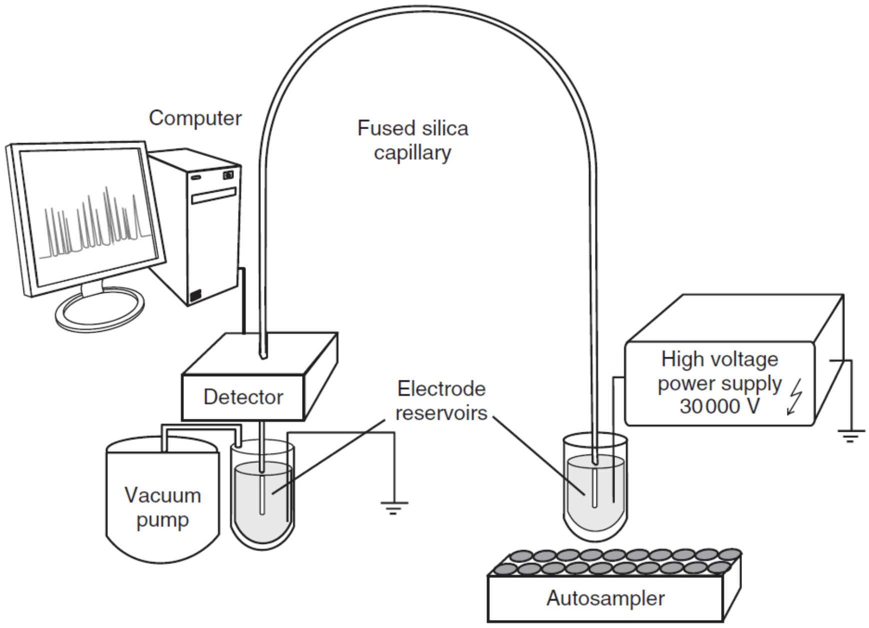
NOTES

Free-flow electrophoresis:

In this technique, a thin buffer film between two parallel plates flows under laminar conditions perpendicular to the electrical field. At one end, the sample is injected at a defined spot, and at the other end the fractions are collected through an array of tubings, which leads to a 96-well microtiter plate. This is the only continuous electrophoretic separation method. The sample components are deflected perpendicular to the flow depending on their charges; they reach the end of the separation chamber at different though stable positions (Figure 14.3.2).



14.3.2 Schematic drawing of a continuous free-flow electrophoresis system



14.3.3 Schematic representation of the set-up of capillary electrophoresis

Capillary electrophoresis (CE):

In capillary electrophoresis, separation is carried out in a fused silica capillary tube 20–30 cm long and with an internal diameter of 50–100 μm. Both ends of the capillary are immersed in a buffer container into which the electrodes are built (Figure 14.3.3). As the sample migrates through the capillary its components separate and elute from the column at different times. The resulting electropherogram looks similar to a GC or an HPLC chromatogram, providing both qualitative and quantitative information. Only capillary electrophoretic methods receive further consideration in this section. There are several different forms of capillary electrophoresis, each of which has its particular advantages, they are:

- ❖ Capillary zone electrophoresis
- ❖ Micellar electro kinetic capillary chromatography
- ❖ Capillary gel electrophoresis
- ❖ Capillary electrochromatography

Controlling factors

The basic instrumentation for capillary electrophoresis is shown in Figure 14.3.4 and includes a power supply for applying the electric field, anode and cathode compartments containing reservoirs of the buffer solution, a sample vial containing the sample, the capillary tube, and a detector. Each part of the instrument receives further consideration in this section.

Capillary tubes:

Most capillary tubes are made from fused silica coated with a 15–35 μm layer of polyimide to give it mechanical strength. The inner diameter is typically 25–75 μm—smaller than the internal diameter of a capillary GC column—with an outer diameter of 200–375 μm The capillary column's narrow opening and the thickness of its walls are important. When an electric field is applied to the buffer solution within the capillary, current flows through the capillary. This current leads to the release of heat called Joule heating. The amount of heat released is proportional to the capillary's radius and the magnitude of the electrical field. Joule heating is a problem because it changes the buffer solution's viscosity, with the solution at the center of the capillary being less viscous than that near the capillary walls.

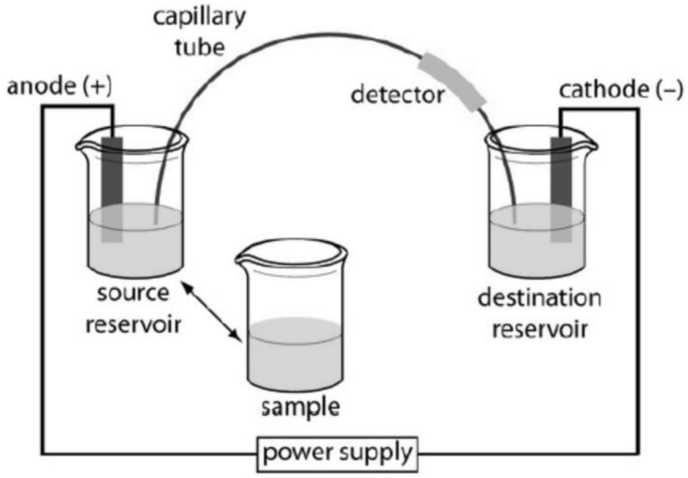


Figure 14.3.4 Schematic diagram of the basic instrumentation for capillary electrophoresis.

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Injecting the sample:

There are two commonly used method for injecting a sample into a capillary electrophoresis column: hydrodynamic injection and electrokinetic injection. In both methods the capillary tube is filled with the buffer solution. One end of the capillary tube is placed in the destination reservoir and the other end is placed in the sample vial.

Hydrodynamic injection uses pressure to force a small portion of sample into the capillary tubing. A difference in pressure is applied across the capillary by either pressurizing the sample vial or by applying a vacuum to the destination reservoir.

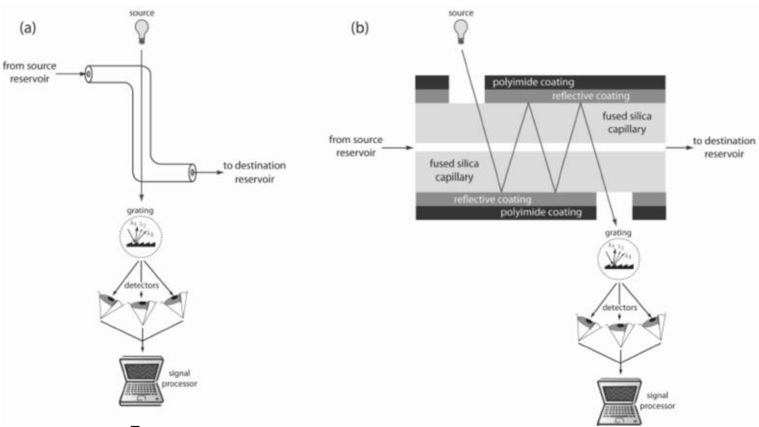
In an electro kinetic injection we place both the capillary and the anode into the sample and briefly apply an potential. The volume of injected sample is the product of the capillary's cross sectional area and the length of the capillary occupied by the sample.

When an analyte's concentration is too small to detect reliably, it may be possible to inject it in a manner that increases its concentration in the capillary tube. This method of injection is called stacking. Stacking is accomplished by placing the sample in a solution whose ionic strength is significantly less than that of the buffer in the capillary tube. Because the sample plug has a lower concentration of buffer ions, the effective field strength across the sample plug, E′ is larger than that in the rest of the capillary.

Detectors:

Most of the detectors used in HPLC also find use in capillary electrophoresis. Among the more common detectors are those based on the absorption of UV/Vis radiation, fluorescence, conductivity, amperometry, and mass spectrometry. Whenever possible, detection is done "on-column" before the solutes elute from the capillary tube and additional band broadening occurs.

UV/Vis detectors are among the most popular. Because absorbance is directly proportional to path length, the capillary tubing's small diameter leads to signals that are smaller than those obtained in HPLC. Several approaches have been used to increase the pathlength, including a Z-shaped sample cell and multiple reflections (see Figure 14.3.5). Detection



limits are about 10^{-7} M.

14.3.5 Two approaches to on-column detection in capillary electrophoresis using a UV/Vis diode array spectrometer: (a) Z-shaped bend in capillary, and (b) multiple reflections

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Ionic
Separations

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Better detection limits are obtained using fluorescence, particularly when using a laser as an excitation source. When using fluorescence detection a small portion of the capillary's protective coating is removed and the laser beam is focused on the inner portion of the capillary tubing. Emission is measured at an angle of 90° to the laser. Because the laser provides an intense source of radiation that can be focused to a narrow spot, detection limits are as low as 10^{-16} M.

Evaluation:

When compared to GC and HPLC, capillary electrophoresis provides similar levels of accuracy, precision, and sensitivity, and a comparable degree of selectivity. The amount of material injected into a capillary electrophoretic column is significantly smaller than that for GC and HPLC—typically 1 nL versus 0.1 μL for capillary GC and 1–100 μL for HPLC. Detection limits for capillary electrophoresis, however, are 100–1000 times poorer than that for GC and HPLC.

The most significant advantages of capillary electrophoresis are improvements in separation efficiency, time, and cost. Capillary electrophoretic columns contain substantially more theoretical plates ($\approx 10^6$ plates/m) than that found in HPLC ($\approx 10^5$ plates/m) and capillary GC columns ($\approx 10^3$ plates/m), providing unparalleled resolution and peak capacity. Separations in capillary electrophoresis are fast and efficient. Furthermore, the capillary column's small volume means that a capillary electrophoresis separation requires only a few microliters of buffer solution, compared to 20–30 mL of mobile phase for a typical HPLC separation.

14.4 DI-ELECTROPHORESIS

Principle

Dielectrophoresis (DEP) is a phenomenon in which a force is exerted on a dielectric particle when it is subjected to a non-uniform electric field (Figure 14.4.1). This force does not require the particle to be charged. All particles exhibit dielectrophoretic activity in the presence of electric fields. However, the strength of the force depends strongly on the medium and particles electrical properties, on the particles shape and size, as well as on the frequency of the electric field. Consequently, fields of a particular frequency can manipulate particles with great selectivity. This has allowed, for example, the separation of cells or the orientation and manipulation of nanoparticles and nanowires. Furthermore, a study of the change in DEP force as a function of frequency can allow the electrical (or electrophysiological in the case of cells) properties of the particle to be elucidated.

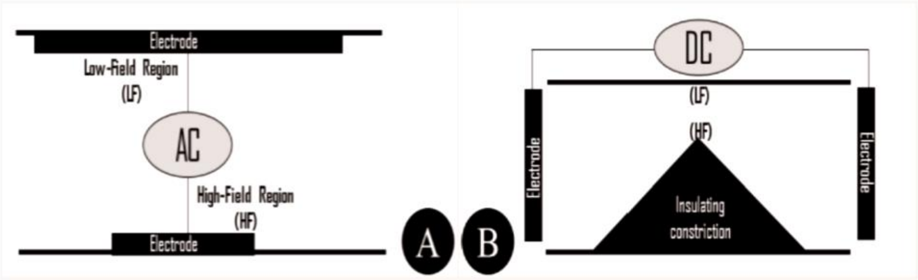


Figure 14.4.1 Generation of non-uniform electric fields for dielectrophoretic applications

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Properties

Dielectrophoresis occurs when a polarizable particle is suspended in a non-uniform electric field. The electric field polarizes the particle, and the poles then experience a force along the field lines, which can be either attractive or repulsive according to the orientation on the dipole. Since the field is non-uniform, the pole experiencing the greatest electric field will dominate over the other, and the particle will move. The orientation of the dipole is dependent on the relative polarizability of the particle and medium, in accordance with Maxwell–Wagner–Sillars polarization. Since the direction of the force is dependent on field gradient rather than field direction, DEP will occur in AC as well as DC electric fields; polarization (and hence the direction of the force) will depend on the relative polarizabilities of particle and medium.

If the particle moves in the direction of increasing electric field, the behavior is referred to as positive DEP (sometime pDEP), if acting to move the particle away from high field regions, it is known as negative DEP (or nDEP). As the relative polarizabilities of the particle and medium are frequency-dependent, varying the energizing signal and measuring the way in which the force changes can be used to determine the electrical properties of particles; this also allows the elimination of electrophoretic motion of particles due to inherent particle charge. Phenomena associated with dielectrophoresis are electrorotation and traveling wave dielectrophoresis (TWDEP). These require complex signal generation equipment in order to create the required rotating or traveling electric fields, and as a result of this complexity have found less favor among researchers than conventional dielectrophoresis.

Controlling factors

Electrode geometries:

At the start, electrodes were made mainly from wires or metal sheets. Nowadays, the electric field in DEP is created by means of electrodes which minimize the magnitude of the voltage needed. This has been possible using fabrication techniques such as

photolithography, laser ablation and electron beam patterning. These small electrodes allow the handling of small bioparticles. The most used electrode geometries are isometric, polynomial, interdigitated, and crossbar.

Isometric geometry is effective for particle manipulation with DEP but repelled particles do not collect in well-defined areas and so separation into two homogeneous groups is difficult. Polynomial is a new geometry producing well defined differences in regions of high and low forces and so particles could be collected by positive and negative DEP. This electrode geometry showed that the electrical field was highest at the middle of the inter-electrode gaps. Interdigitated geometry comprises alternating electrode fingers of opposing polarities and is mainly used for dielectrophoretic trapping and analysis. Crossbar geometry is potentially useful for networks of interconnects.

DEP-well electrodes:

These electrodes were developed to offer a high-throughput yet low-cost alternative to conventional electrode structures for DEP. Rather than use photolithographic methods or other microengineering approaches, DEP-well electrodes are constructed from stacking successive conductive and insulating layers in a laminate, after which multiple "wells" are drilled through the structure. If one examines the walls of these wells, the layers appear as interdigitated electrodes running continuously around the walls of the tube. When alternating conducting layers are connected to the two phases of an AC signal, a field gradient formed along the walls moves cells by DEP.

DEP-wells can be used in two modes; for analysis or separation. In the first, the dielectrophoretic properties of cells can be monitored by light absorption measurements: positive DEP attracts the cells to the wall of the well, thus when probed with a light beam the well the light intensity increases through the well. The opposite is true for negative DEP, in which the light beam becomes obscured by the cells. Alternatively, the approach can be used to build a separator, where mixtures of cells are forced through large numbers (>100) of wells in parallel; those experiencing positive DEP are trapped in the device whilst the rest are flushed. Switching off the field allows release of the trapped cells into a separate container. The highly parallel nature of the approach means that the chip can sort cells at much higher speeds, comparable to those used by MACS and FACS.

Dielectrophoresis field-flow fractionation:

The utilization of the difference between dielectrophoretic forces exerted on different particles in nonuniform electric fields is known as DEP separation. The exploitation of DEP forces has been classified into two groups: DEP migration and DEP retention. DEP migration uses DEP forces that exert opposite signs of force on different particle types to attract some of the particles and repel others. DEP retention uses the balance between DEP and fluid-flow forces. Particles experiencing repulsive and weak attractive DEP forces are eluted by fluid flow, whereas particles experiencing strong attractive DEP forces are trapped at electrode edges against flow drag.

Dielectrophoresis field-flow fractionation (DEP-FFF) is a family of chromatographic-like separation methods in which forces are combined with drag flow to fractionate a sample of different types of particles. Particles are injected into a carrier flow that passes through the separation chamber, with an external separating force (a DEP force) being applied perpendicular to the flow. By means of different factors, such as diffusion and steric, hydrodynamic, dielectric and other effects, or a combination thereof, particles (<1 μm in diameter) with different dielectric or diffusive properties attain different positions away from the chamber wall, which, in turn, exhibit different characteristic concentration profile. Particles that move further away from the wall reach higher positions in the parabolic velocity profile of the liquid flowing through the chamber and will be eluted from the chamber at a faster rate.

Optical dielectrophoresis:

The use of photoconductive materials (for example, in lab-on-chip devices) allows for localized inducement of dielectrophoretic forces through the application of light. In addition, one can project an image to induce forces in a patterned illumination area, allowing for some complex manipulations. When manipulating living cells, optical dielectrophoresis provides a non-damaging alternative to optical tweezers, as the intensity of light is about 1000 times less.

Applications

- DEP is mainly used for characterising cells measuring the changes in their electrical properties. To do this, many techniques are available to quantify the dielectrophoretic response, as it is not possible to directly measure the DEP force. These techniques rely on indirect measures, obtaining a proportional response of the strength and direction of the force that needs to be scaled to the model spectrum.
- Dielectrophoresis can be used to manipulate, transport, separate and sort different types of particles. Since biological cells have dielectric properties, dielectrophoresis has many medical applications. Instruments that separate cancer cells from healthy cells have been made. Platelets have been separated from whole blood with a DEP-activated cell sorter.
- Dielectrophoresis can be used to manipulate, transport, separate and sort different types of particles. DEP is being applied in fields such as medical diagnostics, drug discovery, cell therapeutics, and particle filtration.
- DEP has been also used in conjunction with the semiconductor chip technology for the development of DEPArray technology for the simultaneous management of thousands of cells in a microfluidic device. Single microelectrodes on the floor of a flow cell are managed by the CMOS chip to form thousands of “Dielectrophoretic Cages”, each capable of capturing and moving one single cell under control of a routing software.
- The most effort in studying DEP has been directed towards satisfying the unmet needs in the biomedical sciences.

14.5 Electrodialysis

First commercial equipment based on Electrodialysis (ED) technology was developed in the 1950s to demineralize brackish water (Juda & McRae, 1950; Winger et al. 1953). Since then ED has advanced rapidly because of improved ion exchange membrane properties, better materials of construction and advances in technology. In the 1960s, Electrodialysis Reversal (EDR) was introduced, to avoid organic fouling problems (Mihara & Kato, 1969). Over the past twenty years EDR has earned a reputation as a membrane desalination process that works economically and reliably on surface water supplies, reuse water and some specific industrial applications when designed and operated properly.

Principle

ED is an electrochemical separation process in which ions are transferred through ion exchange membranes by means of a direct current (DC) voltage. The process uses a driving force to transfer ionic species from the source water through cathode (positively charged ions) and anode (negatively charged ions) to a concentrate wastewater stream, creating a more dilute stream (Figure 14.5.1).

ED selectively removes dissolved solids, based on their electrical charge, by transferring the brackish water ions through a semi permeable ion exchange membrane charged with an electrical potential. It points out that the feed water becomes separated into the following three types of water:

- ✓ product water, which has an acceptably low conductivity and TDS level;
- ✓ brine, or concentrate, which is the water that receives the brackish water ions; and
- ✓ electrode feed water, which is the water that passes directly over the electrodes that create the electrical potential.

EDR is a variation on the ED process, which uses electrode polarity reversal to automatically clean membrane surfaces. EDR works the same way as ED, except that the polarity of the DC power is reversed two to four times per hour. When the polarity is reversed, the source water dilute and concentrate compartments are also reversed and so are the chemical reactions at the electrodes. This polarity reversal helps prevent the formation of scale on the membranes.

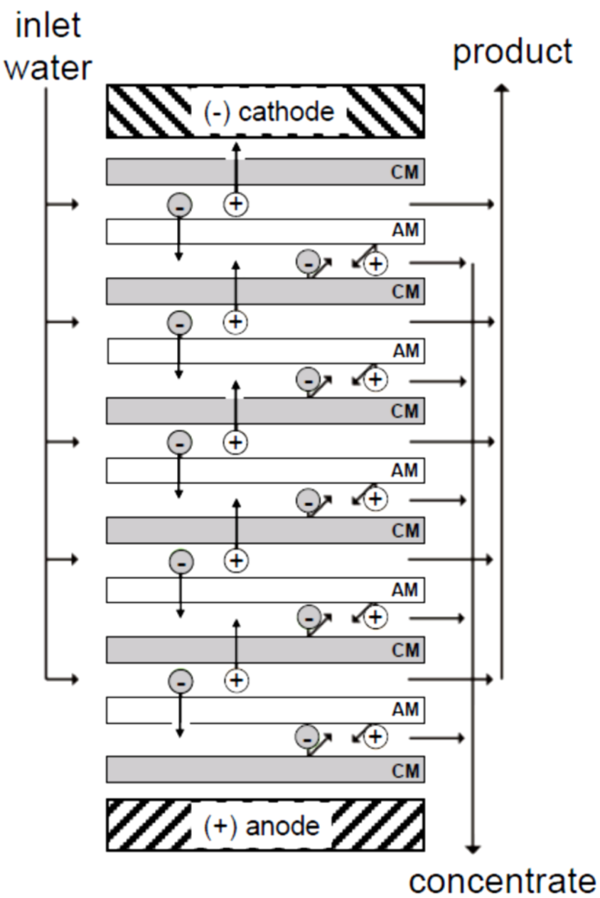


Figure 14.5.1 Illustration of principles of ED

Controlling factors

Membrane stacks:

All ED and EDR systems are designed specifically for a particular application. The number of ions to be removed is determined by the configuration of the membrane stack. A membrane stack may be oriented in either a horizontal or vertical position.

Cell pairs form the basic building blocks of an EDR membrane stack (Figure 1). Each stack assembled has the two electrodes and groups of cell pairs. The number of cell pairs necessary to achieve a given product water quality is primarily determined by source water quality, and can design stacks with more than 600 cell pairs for industrial applications.

A cell pair consists of the following:

- ❖ • Anion permeable membrane
- ❖ • Concentrate spacer
- ❖ • Cation permeable membrane
- ❖ • Dilute stream spacer

In each stack, we can observe different flows:

a) 1. Source water (feed) flows parallel only through demineralizing compartments, whereas the concentrate stream flows parallel only through concentrating compartments.
b) 2. As feed water flows along the membranes, ions are electrically transferred through membranes from the demineralized stream to the concentrate stream.
c) 3. Flows from the two electrode compartments do not mix with other streams. A de-gasifier vents reaction gases from the electrode waste stream.
d) 4. Top and bottom plates are steel blocks that compress the membranes and spacers to prevent leakage inside the stack.

Effluent from these compartments may contain oxygen, hydrogen, and chlorine gas. Concentrate from the electrode stream is sent to a de-gasifier to remove and safely dispose of any reaction gases.

Membranes:

The membranes are produced in the form of foils composed of fine polymer particles with ion exchange groups anchored by polymer matrix. Impermeable to water under pressure, membranes are reinforced with synthetic fibre which improves the mechanical properties of the membrane. The two types of ion exchange membranes used in electrodialysis are:

- ❖ Cation transfer membranes which are electrically conductive membranes that allow only positively charged ions to pass through. Commercial cation membranes generally consist of crosslinked polystyrene that has been sulfonated to produce $–SO_3H$ groups attached to the polymer, in water this group ionizes producing a mobile counter ion (H^+) and a fixed charge ($-SO^{3-}$).

Ionic Separations

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- Anion transfer membranes, which are electrically conductive membranes that allow only negatively, charged ions to pass through. Usually, the membrane matrix has fixed positive charges from quaternary ammonium groups (-NR3+OH-) which repel positive ions. Both types of membranes show common properties: low electrical resistance, insoluble in aqueous solutions, semi-rigid for ease of handling during stack assembly, resistant to change in pH from 1 to 10, operate temperatures in excess of 46 ºC, resistant to osmotic swelling, long life expectancies, resistant to fouling and hand washable.

The membranes are permselective (or ion selective) that refers to their ability to discriminate between different ions to allow passage or permeation through the membrane. In these sense membranes can be tailored to inhibit the passage of divalent anions or cations, such as sulfates, calcium, and magnesium. For example, some membranes show good permeation or high transport numbers for monovalent anions, such as Cl^- or NO^{3-}, but have low transport numbers and show very low permeation rates for divalent or trivalent ions, such as SO_4^{-2}, PO_4^{-3}, or similar anions. This is achieved by specially treating the anion membrane, and the effect can be exploited to separate various ions. The relative specificities vary, with the monovalent anion membrane showing the greatest specificity, for example, the ratio of chloride to sulfate ion transport numbers.

Spacers:

The spaces between the membranes represent the flow paths of the demineralized and concentrated streams formed by plastic separators which are called demineralized and concentrate water flow spacers respectively. These spacers are made of polypropylene or low density polyethylene and are alternately positioned between membranes in the stack to create independent flow paths, so that all the demineralized streams are manifolded together and all the concentrate streams are manifolded together too. Demineralizing and concentrating spacers are created by rotating an identical spacer 180°. Demineralizing spacers allow water to flow across membrane surfaces where ions are removed, whereas concentrating spacers prevent the concentrate stream from contaminating the demineralized stream.

There is a spacer design with a "tortuous path" in which the spacer is folded back upon itself and the liquid flow path is much longer than the linear dimensions or the unit. Another

kind of spacers is a "sheet flow" that consists of an open frame with a plastic screen separating the membranes. These spacers are operated at lower flow velocities, to achieve a degree of desalting in each pass through the stack, comparable to the tortuous path or sheet flow spacers. In general the increase of turbulence promotes mixing of the water, use of the membrane area, and the transfer of ions. Turbulence resulting from spacers also breaks up particles or slime on the membrane surface and attracts ions to the membrane surface. Flow velocity ranges from (18 to 35 cm/s, creating a pressure drop between the inlet and outlet.

Different models and sizes of spacers satisfy specific design applications. The main difference in spacer models is the number of flow paths, which determines water velocity across the membrane stack and contact time of the source water with the membrane. Since water velocity is responsible for the degree of mixing and the amount of desalting that occurs across membranes, velocity is an important design parameter for spacer choice. Because the same spacers are used for both demineralized and concentrated water in EDR systems, the flow rates of both these streams should be equalized to prevent high differential pressures across the membranes.

Electrodes:

A metal electrode at each end of the membrane stack conducts DC into the stack. Electrode compartments consist of an electrode, an electrode water-flow spacer, and a heavy cation membrane. The electrode spacer is thicker than a normal spacer, which increases water

velocity to prevent scaling. This spacer also prevents the electrode waste from entering the main flow paths of the stack. Because of the corrosive nature of the anode compartments, electrodes are usually made of titanium and plated with platinum. Its life span is dependent on the ionic composition of the source water and the amperage applied to the electrode. Large amounts of chlorides in the source water and high amperages reduce electrode life. Polarity reversal (as in EDR) also results in significantly shorter electrode lifetimes than for non-reversing systems.

Operation:

When DC potential is applied across the electrodes, the following take place

At the cathode, or negative electrode (-):

- Cations (Na^+) attraction
- Pairs of water molecules break down (dissociate) at the cathode to produce two hydroxyl (OH^-) ions plus hydrogen gas (H_2). Hydroxide raises the pH of the water, causing calcium carbonate ($CaCO_3$) precipitation.

And at the anode, or positive electrode (+):

- Anions (Cl^-) attraction
- Pairs of water molecules dissociate at the anode to produce four hydrogen ions (H^+), one molecule of oxygen (O2), and four electrons (e–). The acid tends to dissolve any calcium carbonate present to inhibit scaling.
- Chlorine gas (Cl_2) may be formed.

Colloidal particles or slimes that are slightly electronegative may accumulate on the anion membrane and cause membrane fouling. This problem is common to all classes of ED systems. These fouling agents are removed by flushing with cleaning systems. In EDR systems, the polarity of the electrodes is reversed two to four times each hour. When polarity is reversed, chemical reactions at the electrodes are reversed. Valves in the electrode streams automatically switch flows in the two types of compartments. Streams that were in demineralizing compartments become concentrate streams, and concentrate streams become demineralizing streams.

Design:

In commercial practice, the basic apparatus for ED/EDR is a stack of rectangular membranes terminated on each end by an electrode. Flow of the process streams is contained and directed by spacers that alternate with the membranes. The membranes are arranged alternately cation and anion. The assembly of membrane spacers and electrodes is held in compression by a pair of end plates. The apparatus thus resembles a plate-and-frame filter press. Stack is completed with pumps, piping and an electrical sub-system that includes: adjustable DC power supply, rectifiers to convert alternating current (AC) power to DC power, internal control system with controls, reversal timing (only for EDR), and alarms.

Applications:

- Over the last ten to fifteen years, numerous advances in membrane and system technology have made EDR an especially attractive technology, both in terms of performance and cost effectiveness.
- Desalting process is applied mainly to brackish water process, tertiary wastewater production and specific industrial applications.
- EDR could be in a near future the technology of choice for many applications because its efficiency to desalt water needed in different fields like drinking water, reuse water and many industrial applications, like food, beverages and mining among others.
- In electrodialysis, instead of large protein and DNA molecules, soluble and smaller charged ions migrate between concentrated and dilute flows through two distinct and oppositely charged membranes that only allow for a single type of ion migration (positive or negative). As a result, one can obtain dilute streams with small amounts of ion, and two types of concentrate streams with the positive and negative ion respectively.
- It is a modern process that is competitive to reverse osmosis and is gaining attention from the pharmaceutical and food industries. It has a number of important advantages such as preservation of nutritious properties of the product and lower energy consumption.

Check Your Progress – 4

1. What is the principle of Electrophoresis?
2. Mention some uses of dielectrophoresis (DEP)
3. Describe two types of membranes used in Electrodialysis.

14.6 ANSWER TO CHECK YOUR PROGRESS QUESTION

1. Electrophoresis is a class of separation techniques in which the analytes get separated by their ability to move through a conductive medium, usually an aqueous buffer, in response to an applied electric field.
2. DEP is mainly used for characterising cells measuring the changes in their electrical properties. Dielectrophoresis can be used to manipulate, transport, separate and sort different types of particles.

3. DEP is being applied in fields such as medical diagnostics, drug discovery, cell therapeutics, and particle filtration.
4. Cation transfer membranes which are electrically conductive membranes that allow only positively charged ions to pass through whereas Anion transfer membranes, which are electrically conductive membranes that allow only negatively, charged ions to pass through.

14.7 SUMMARY

Ionic separation is a technique which involves the movement of ions between two electrodes that tend to travel towards the electrodes that bear opposite charges. Depending on the kind of the molecule charge they carry they move either to anode or cathode. Electrophoresis is a method of separation where in charged molecule migrate in differential speed in an applied electric field. Electrodialysis is the ion exchange membrane separation process in which the electric potential is used as the driving force for the separation. It consumes only low energy and the higher brine concentration is achievable. It is used in waste water process treatment, table salt production, boiler feed water production etc.

14.8 KEY WORDS

- Capillary electrophoresis
- Dielectrophoresis
- membranes
- Electrodialysis

14.9 SELF-ASSESSMENT QUESTION AND EXERCISES

1. Discuss about the types of electrophoresis.
2. Explain the process of Dielectrophoresis
3. Give some applications of Electrodialysis

14.10 FURTHER READINGS

1. King, C.J. Separation Processes, Tata McGraw - Hill Publishing Co., Ltd., 1982.
2. Ronald W.Roussel - Handbook of Separation Process Technology, John Wiley, New York, 1987.

Sub.Code:344401

DISTANCE EDUCATION
M.Sc., (Chemistry) DEGREE EXAMINATION
Analytical Chemistry
(CBCS -2018-19 Academic Year onwards)

Time : Three hours **Maximum : 75 marks**

Part-A (10 x 2=20)

Answer **all** questions

1. What is absolute error?
2.
3. Define standard deviation.
4. Give some applications of Ion-selective electrodes.
5. Explain electro transfer reactions in cyclic voltammetry.
6. Explain the principle of paper chromatography.
7. What does the choice of filter paper depend on?
8. What is ionization and its types?
9. What is the principle of size exclusion chromatography?
10. Define sublimation
11. What is mean by capillary electrophoresis?

Part-B (5 x 5=25)

Answer **all** questions choosing either (a) or (b)

12. (a) Explain the classification of errors.

(or)

(b) What is significant figure and write the rules to determine the significant figures?

13. (a) Explain potentiometric methods

(or)

(b) Differentiate the kinds of polarography.

13. (a) Describe AC polarography

(or)

(b) Differentiate between chronoamperometry and chronopotentiometry

14. (a) Describe principle and instrumentation of TLC

(or)

(b) write down the application of Gas chromatography

15. (a) Discuss the instrumentation of gel permeation chromatography

(or)

(b) write about the controlling factors involved in electrodialysis

Part-C (3 x 10 =30)

Answer any **three** questions.

16. Explain about F-Test and Q-Test?
17. (a) Discuss about the types of cells involved in polarographic reactions
    (b) Describe about Dropping mercury electrode (DME)
18. (a) Explain the principle and theory behind GC-MS
    (b) write about the significant features and types of HPLC
19. Schematically explain the purification method of fractional crystallization and sublimation process.
20. Briefly explain Electrophoresis.